STUDY ON ISOLATION AND IDENTIFICATION OF FUNGAL AGENTS IN PATIENTS WITH CORNEAL ULCER IN TERTIARY CARE OPHTHALMIC HOSPITAL

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CERTIFICATE

This is to certify that this dissertation entitled **" STUDY ON ISOLATION AND IDENTIFICATION OF FUNGAL AGENTS IN PATIENTS WITH CORNEAL ULCER IN TERTIARY CARE OPHTHALMIC HOSPITAL"** is the bonafide original work done by **Dr Dr.M.R.VASANTHAPRIYAN,** Post graduate in Microbiology, under my overall supervision and guidance in the department of Microbiology, Govt. Kilpauk Medical College, Chennai, in partial fulfillment of the regulations of The Tamil Nadu Dr. M.G.R. Medical University for the award of **M.D Degree in Microbiology (Branch IV).**

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DECLARATION

I solemnly declare that this dissertation **" STUDY ON ISOLATION AND IDENTIFICATION OF FUNGAL AGENTS IN PATIENTS WITH CORNEAL ULCER IN TERTIARY CARE OPHTHALMIC HOSPITAL"** is the bonafide work done by me at the Department of Microbiology, Govt. Kilpauk Medical College and Hospital, Chennai, under the guidance and supervision of **Prof. Dr.THYAGARAJAN RAVINDER, M.D.,** Professor and Head, Department of Microbiology, Govt. Kilpauk Medical College, Chennai-10.

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Date :

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CONTENTS

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Introduction

INTRODUCTION

The eye is an important organ of sensory perception. [50] The Cornea is a clear, circular, transparent structure and continuous with the sclera, the junction between the two is called the limbus.

Microscopically the cornea consists of five layers, [27] [28]

(a) The corneal epithelium with its basement membrane

(b) The Bowman's layer

(c) The stroma

(d) The Descemet's membrane

(e) The Endothelium

The cornea is usually kept free from microbial invasion due to the intact epithelium and cleansing effect of the tears. Corneal ulceration is defined as any disruption to the intact epithelium with underlying stromal infiltration and suppuration associated with signs of inflammation,[17] the organism either being implanted from without or from conjuctival flora.[83] Exception to the rule are Neisseria gonorrhea and Corynebacterium diphtheria which are able to invade an intact epithelium.[41]

The possible reasons for the corneal ulceration are,

(a) Trauma

(b) Infection , which again may be organism or may be an extension of the disease process from other ocular tissue

(c) Allergic conjunctivitis

(d) Autoimmune disorders.

Conditions like trauma, steroid therapy and immunosuppressive states like Diabetes mellitus render the cornea susceptible to bacterial, fungal, parasitic infections. [8]

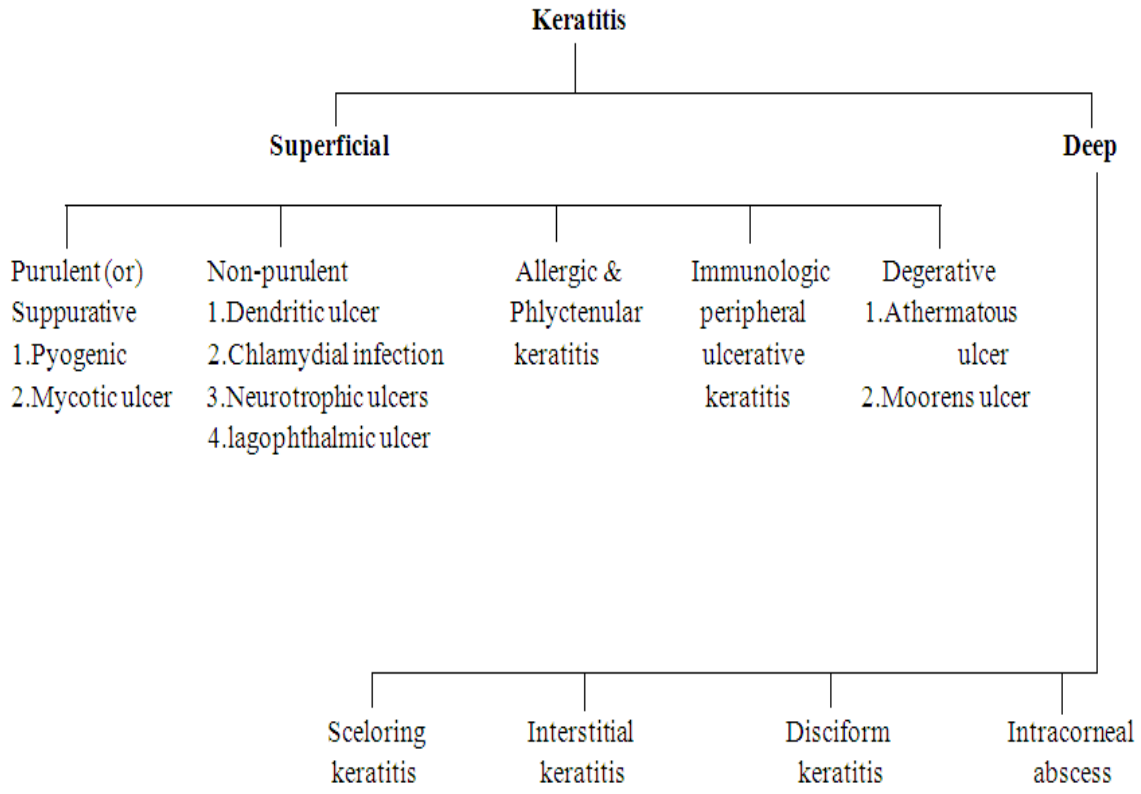
Any organism has the potential to cause microbial keratitis and corneal ulceration, given the appropriate condition and predisposing risk factors. A wide spectrum of microbial organism like bacteria, virus, fungus and parasite can produce infectious corneal ulcer. The fungal isolates commonly associated with infectious corneal ulceration are Aspergillus species, Penicillium species and Fusarium species. The commonly encountered aetiological agent of fungal corneal ulcer shows wide geographical variation. [51]. This phenomenon form the base for varying susceptibility profile among the fungal agents.

Break down of corneal defence mechanism will allow entry of microorganism to lodge in the corneal stroma. Activation of complement and release of mycotoxins result in suppurative corneal ulceration. [116]

Signs of established infections of the cornea include bulbar injection, corneal ulcerations, hypopyon formation, iritis and extensive posterior segment involvement if not treated.

Adhesion, entry and multiplication of organism in a compromised cornea lead to release of chemotactic factors and toxins and accumulation of polymorphs. If infection is detected early and treated promptly before it spreads to involve the descemets membrane and endothelium, tissue repair and healing occurs. Otherwise the usually tough descemets membrane gives way and this may result in a corneal perforation with attendant complications. Organisms produce extracellular toxins which kill or damage corneal epithelium and stroma, thus enhancing the adherence of organism to the tissues. [118]

Keratitis may be classified as superficial and deep. [27]



Corneal ulcer is an ocular emergency that require prompt management to ensure the best visual outcome for the patients. A clinical diagnosis doesn't give an unequivocal indication of the causative organism because of wide range of organism can produce a similar clinical picture. Therefore microbiological evaluation plays a significant role in the diagnosis and treatment of corneal ulcer.

Direct microscopic evaluation of smears provide immediate information about the causative organism and is helpful in starting antimicrobial therapy in a short course of time and the aetiological agents confirmed and isolated by culture which is a gold standard method in the diagnosis.[9]

Corneal inflammation is considered as an emergency and an event of threat to vision, on the basis of virulence of the organism by means of rapid progression, toxin production and invasion property in relation to patient immune status. Empirical therapy is necessary based on preliminary testing and switch on to specific therapy with antimicrobial susceptibility testing.

The incidence of infectious keratitis has increased in the last few years due to the improvement in microbiological diagnostic technique and introduction of the therapeutic measures such as wide spread use of broad spectrum antibiotics , antifungal and immunosuppressive drugs.[10]

Corneal blindness is a major public health problem worldwide and infectious keratitis is one of the predominant preventable cause. [10]

In south East Asia according to an estimate 6.5 million people are affected and 1.3 million become blind due to infectious corneal ulcer every year. [64] Corneal ulceration is common in South India and often occurs after a superficial corneal injury with organic material.

Mostly the fungal pathogens are opportunistic, they show wide range of resistance to antifungal agents and not able to overcome the problem with currently available antifungal agents. So antimicrobial susceptibility tests are mandatory to monitor the efficiency of available antimicrobial agents and the emergence of drug resistance among the fungus causing corneal ulceration.

Considering the importance of corneal ulceration and its impact on vision, the present study is conducted to identify the predisposing factors of fungal corneal ulcers and the aetiological agents and antifungal susceptibility pattern, in patients attending a tertiary care Ophthalmic Hospital in Chennai.

Review of Literature

REVIEW OF LITERATURE

Historical review of organism causing ocular infections:

The first textbook on eye diseases was written by Antonio Scarpa in 1801 and was translated to English in 1806. [41] During 1851 -1856, Arlt established the etiology and manifestation of eye diseases.

In 1879 , Leber proved the microbial etiology of keratomycosis by demonstrating the causative fungus in direct smear, culture and experimental studies and credited with documenting the first case of fungal keratitis caused by Aspergillus glucus in a agricultural worker.[82][98]

Microbiology of the eye and its infections:

Corneal ulceration in the developing world is a silent epidemic and leading cause of ocular morbidity and blindness worldwide.

The aetiological agents involved in infectious corneal ulcer can be classified as Bacterial, Fungal, Viral, Protozoal.[17]

The Fungal agents are, [94][38]

(i) Hyaline hypomycetes:

(a) Aspergillus species

(b) Acremonium species

(c) Penicillium species

(d) Fusarium species

(e) Pseudallescheria species

(ii) Phaeoid hypomycetes:

(a) Aureobasidium pullulans

(b) Alternaria species

(c) Bipolaris species

(d) Curvularia species

(e) Cladosporium species

(iii)Yeast like fungi:

(a) Candida albicans

(b) Candida krusei

(c) Candida tropicalis

Mycotic corneal ulceration is reported as a major cause of keratitis in tropical regions including India. [71] Srinivasan *et al* reported that Mycotic keratitis responsible for more than 50% of all cases of ocular mycosis. [88]

The list of fungal species associated with corneal ulceration was very long however a few species are seen to be more aggressive opportunistic fungi and account for the majority of infections recorded. [72]

Studies on microbial infection of the eye are increasing in respect with reducing the mortality and morbidity due to ocular emergencies. The presence of fungi in the cornel ulcer seems to vary not only from place to place but also with relation to the occupation. [90][29]

Particularly people working with the decaying vegetation like mouldy hay in agriculture were more prone to develop infectious corneal ulcer.[45]

Minor trauma to corneal epithelium leads to direct implantation of fungal spores leading to corneal ulcer.[52][73] This condition apparently occur more frequently in developing countries than in the developed world.[91]

Aspergillus species are hyaline saprophyte filamentous fungi grow readily on Sabouraud's dextrose agar.[91] Aspergillus species commonly associated with fungal corneal ulcer in relation to vegetative matter. The colonies of Aspergillus fumigatus are velvety or powdery, smoky green with white to tan reverse. The Conidiophore is smooth with uniseriate phialides covering upper half of the vesicle. The colonies of Aspergillus flavus are velvety, yellow to green in colour. The phialides are uniseriate or biseriate but cover the entire vesicle. The colonies of Aspergillus niger are wooly at first, white to yellow then turning dark black later. Phialides are biseriate covering the entire vesicle.

Fusarium colonies are pluffy to cottony owing to extensive mycelium and diffusible pigment is produced on reverse. Conidia are produced singly or in conidial balls, hyaline and unicellular or transversly septate. Microconidia are single celled and Macroconidia are oblongate and cylindrical shows bean or cresent shaped.

Fusarium species have also been found to be the principle fungal pathogen in Florida, Peruguay, Singapore, Nigeria, Tanzania and Hong Kong. This phenomenon may be explained by differences in climate and natural environment.

Curvularia shows rapidly growing, floccose, and brown with black on reverse. The conidiophores are simple, bearing conidia apically. Conidia are transversly septate and cylindrical or slightly curved. Gopinathan *et al* in 2002 and Garg *et al* in 2000 and Leck *et al* in 2002 reported that Curvularia to be the third most important cause of keratitis.

Acremonium Colonies are usually slow growing, often compact and moist at first, becoming powdery, suede-like or floccose with age, and may be white, grey, pink, rose or orange in color. Hyphae are fine and hyaline and produce mostly simple awl-shaped erect phialides. Conidia are usually one-celled (ameroconidia), hyaline or pigmented, globose to cylindrical, and mostly aggregated in slimy heads at the apex of each phialide. [62]

Jagadish chandar *et al* in 1993 reported 8% fungal corneal ulcer were caused by Acremonium species in Chandigarh.[40] Namrata kumara *et al* in 2002 studied mycotic keratitis in Patna documented 3.94% of Acremonium species were isolated.[62]

Penicillium Colonies are usually fast growing, in shades of green, sometimes white, mostly consisting of a dense felt of conidiophores. Microscopically, chains of single-celled conidia (ameroconidia) are produced in basipetal succession from a specialized conidiogenous cell called a phialide. The term basocatenate is often used to describe such chains of conidia where the youngest conidium is at the basal or proximal end of the

chain. In Penicillium, phialides may be produced singly, in groups or from branched metulae, giving a brush-like appearance known as a penicillus.[114]

Verenkar M P *et al* in 1998 study in Goa reported 12.5% of corneal ulcer shows Penicillium species.[100] Namrata kumara *et al* in 2002 studied mycotic keratitis in Patna documented 7.89% of Penicillium species were isolated.[62]

Carmichael *et al* from South Africa studied hundred and ten cases of corneal ulcers of which six proved positive for fungus.

While Ainley and Smith in 1965 , Nema *et al* in 1966 and Srinivasa Rao and K.N.Rao in 1972 have reported that about 25-30% of their study groups carried fungi in their conjuctival sacs, Hammake and Ellis in 1960 reported a lower rate of 10.3% and Duke Elder in 1969 a really very high rate of 83%. However the reporters have investigated individuals who were paddy harvesters and labourers.

Gupta *et al* in 1991 conducted a study on the conjuctival flora of sixty two patients suffering from fungal corneal ulcers and showed fifteen patients (25%) had fungal invasion.

With increasing use of antimicrobial agents, the pattern of normal flora and infecting organisms has undergone changes all over the world. Another interesting feature of microbial etiology of keratitis is its endemicity pattern. This is substantiated by reports of surveys conducted all

over the world. The incidence of fungal keratitis and the species of organism causing them vary from place to place.

In a study conducted by Liesegang and Foster in south florida in 1999[46] involving six hundred and sixty three patients, the fungal isolates contributes 20.1% among the isolates Fusarium species were the most common and Aspergillus was the next pathogen being isolated.[46]

Xie L *et al* 2006[101] studied the epidemiological features, laboratory findings, and treatment outcomes in patients with fungal keratitis in north China state that Fusarium species being the most commonly isolated pathogen in fungal keratitis.

Candida albicans was the most common agent in a study conducted at Willi's eye hospital, Philadelphia in May 2002.[113]

Liesegang and Foster in 1980[46] and Tanure *et al* in 2000[113] published Candida albicans frequently associated with corneal ulceration.

A report of keratomycosis from Winconsin by Chin *et al* , states that Candida species was the most common fungal pathogen isolated,[32] with Aspergillus species being the least common isolate.

In India microbial studies of corneal ulcer have been carried out in various parts.[1] These studies also demonstrated the varying pattern of the organism causing corneal infections. Species of Penicillium, Alternaria, Curvularia, Bipolaris, Acremonium, Aureobasidium were isolated frequently in studies conducted in most part of India and Nepal.

In an extensive study by Upadhyay *et al* in Nepal, [98] Aspergillus species was the predominant fungal pathogen and Fusarium species were less commonly isolated.

Chowdhary *et al* in 2005 study on spectrum of Fungal Keratitis in North India comprise epidemiology and laboratory results of fungus causing corneal ulceration.[12]

Poria *et al* in 1995 from Jhamnagar reported Fusarium species was the predominant fungal isolate.[110]

A study in costal Karnataka in 1992 published Aspergillus fumigatus as the most common fungal pathogen.[50]

Sood *et al* from Pondicherry reported Aspergillus fumigatus commonly isolated from corneal ulcer.[82]

Philip A. Thomas *et al* from Trichirapalli reported Fusarium species was the predominant fungi isolated and Cladosporium and Curvularia species were less commonly isolated.[65]

Savithri Sharma *et al* from Madurai reported Fusarium species showed high prevalence among the isolates.[84]

V.V.Pankajalaksmi *et al* from Madras in her study of keratomycosis states that Aspergillus species were the most common fungus isolated from corneal ulcers. Curvularia, Drechlera, Candida and Penicillium species were the other fungi cultured.[67]

With improved techniques in culture method, immunology and cytology the availability of well documented data has also improved. Trials and authentic use of newer antifungal drugs improves the diagnostic as well as therapeutic advantages.[85]

These regional differences are important clinically because they influence the laboratory method to be used in the isolation of the etiological agents and also the type of initial therapy instituted by the ophthalmologist.

Epidemiology of keratitis:

Age, Sex, Occupation, socioeconomic status and climate play an important role in the causation and distribution of infections of the cornea. Although keratitis is found in all age groups, it predominantly occurs between 30 to 50 yrs of age.[86][66]

Males are more commonly affected than females, but in the agricultural population the incidence may be equal or more in females.[53]

It is seen that occupation and etiology are interrelated, with the sex incidence being an associated finding. Datta L.C *et al* in 1981 reported a lot of variation among fungal species isolated from different workers.[16]

Ocular trauma particularly with vegetative matter is a well known predisposing factor in fungal corneal ulcers.[73][47] Often the traumatic episode that cause break in the epithelium gives a way for the pathogen to enter. The data derived from a retrospective case control study in Singapore suggested

that mycotic keratitis principally caused by Fusarium, Aspergillus were frequently associated with mechanical trauma.[47]

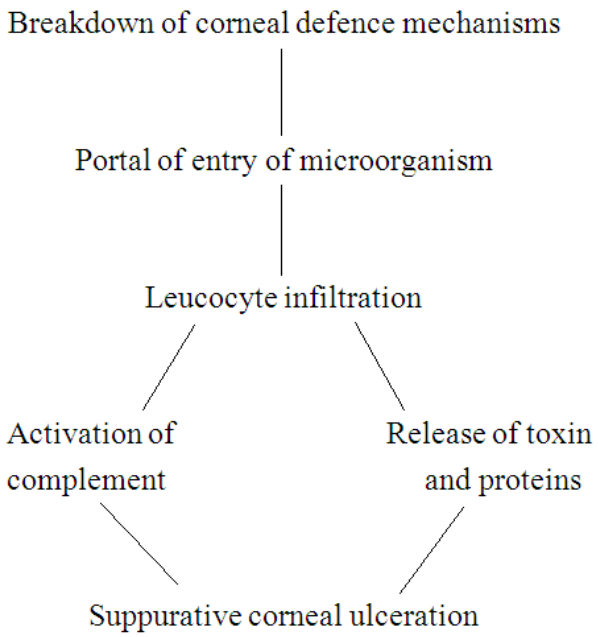
Dry, dusty and windy environment have an increased risk of microtrauma to the cornea, resulting in an increased incidence of fungal keratitis during these seasons.

Dietary deficiencies also play a major role in causing corneal ulcers especially in children in the developing countries.

Contact lens wear appear to be the most important risk factor for development of ulcerative keratitis in developed countries.[18][19]

Other risk factors include use of topical corticosteroids,[10][90] previous ocular surgery,[74] absence of corneal sensation from leprosy and herpetic keratitis,[75] systemic disease like diabetes mellitus.[47] Wong *et al* in 1997 reported 25% of cases of fungal corneal ulcer associated with corticosteroid exposure. An experimental study by Albert P. Ley showed that cortisone applied to traumatized rabbit corneas in the presence of pathogenic fungi resulted in a higher incidence of keratomycosis, thus substsntisting Thygeson's observation.[4] Panda *et al* in 1997 and Gupta *et al* in 1999 documented the corneal ulceration after ocular surgery in two cases out of forty eight cases.

Pathogenesis :



The fungi are unable to penetrate intact corneal epithelium hence any trauma particularly, organic matter facilitate penetration of fugal inoculums into corneal stroma. The fungal hyphae invade from corneal ulcer to stroma. Coagulation necrosis associated with loss of keratocytes and oedematous changes of collagen fibres occur. Satellite lesions are formed around main site of involvement. Late in course of disease process, hyphae may be seen in Descemet's membrane, encased in dense neutrophilic exudates of hypopyon.[37]

They multiply and cause tissue necrosis and elicit inflammatory reaction. They can penetrate the intact descemets membrane and gain access into the anterior chamber or the posterior chamber resulting in the exogenous endophthalmitis.[60] Mycotoxins and proteolytic enzymes of fungi augment the tissue damage.[60] Hogan L.H *et al* in 1996 reviewed the putative

virulence factors of medically important fungi.[34] The cell mediated immunity has clear role in protection against fungal infections.

Clinical manifestations:

Clinical presentation and examination findings are preliminary in the diagnosis of fungal corneal ulcer. The patients generally present with the complaints of pain, watering, redness, photophobia, diminished vision usually presented unilaterally and vision blurred. On examination there may be conjuctival chemosis, congestion, purulent discharge, hypopyon and stromal infiltration.[28][55]

In addition, the presenting clinical features that are specific to fungal ulcers include a greyish white infiltration with feathery margins, rough texture and raised borders with endothelial plaques, satellite lesions [44] and folds in Descemet's membrane. The surrounding corneal stroma is oedematous. The presence of pigmented infiltrate may be an important diagnostic clue for phaeoid fungi.

DIAGNOSTIC TECHNIQUES:

Many fungal ulcers demonstrate no striking morphologic pattern and often it is not possible to differentiate clinically between fungal keratitis with other ocular infections.[21] To determine the causative organism meticulous collection of microbiological specimens of critical importance.[61]

Specimen collection:

Corneal scraping are collected under strict aseptic precautions by an ophthalmologist using sterile No.15 Bard Parker blade[3] after installation of a local anaesthetics like 2% lignocaine hydrochloride from leading edge of the ulcer.[3]

Specimen placed over slide for staining and inoculated in to Sabouraud dextrose agar with Gentamicin[48] and Brain Heart infusion agar with Gentamicin[61] for culture. Denis M.O'Day and association report that BHI broth with Gentamicin is the useful medium for isolation of fungal pathogens from corneal specimens.[20]

MICROSCOPIC EVALUATION OF SMEARS:

A. 10% Potassium Hydroxide mount :

Corneal scrapings were placed in a glass slide with 10% KOH to see the fungal elements.

In 1985 Arafia *et al* reported that KOH mount was an effective and easy method in the detection of fungi compared with other fungal stains and correlate with culture reports.[30]

In 1993 vajpayee *et al* reported that 10% KOH wet mount demonstrate fungal elements in 94.3% of total culture positive cases of keratomycosis.[99]

In 1998 Sharma *et al* reported that 10% KOH mount positive in 100% total culture proven cases.[92]

Chowdhary *et al* in 2005 have concluded that the direct microscopic examination of KOH mount is a rapid, reliable and inexpensive diagnostic modality, which would facilitate the institution of early antifungal therapy before culture reports become available, thus proving to be sight saving.[11]

In 2007 Bharathi *et al* concluded that KOH smear has a greater diagnostic value in the diagnosis of fungal keratitis.

Chandar *et al* in 1993 reported that fungi could be detected in corneal tissue by KOH was 71.4% of culture positive cases.

B.Gram stain:

Smears prepared by corneal scraping and Gram staining done to observe the bacteria and yeast like cell.[61]

Study by Sharma *et al* in 1998, fungus was identified in 86.4% of cases with Gram stain preparation.[92]

Bharathi *et al* in 2006 reported 100% sensitivity of Gram stain procedure in the diagnosis.[9]

C.Calcofluor white stain:

This is a water soluble colourless textile dye and fluorescent whitener. It selectively binds to chitin and cellulose of the fungal cell wall. It fluoresces light blue when expose to UV light (346-365nm).[36]

To the corneal scraping in a slide, 1 drop of 0.1% calcofluor white with 0.1% Evans blue and 1 drop 10% KOH are added. A coverslip is placed over

the specimen and examined under fluorescent microscope. The morphology of smaller fungal elements was better appreciated in calcofluor white mount.

Chandar *et al* in 1993 reported that fungi could be detected in corneal tissue by calcofluor white staining in 95.2% of patients, where KOH mount and culture were positive in 89.6% of patients.

D. Acridine Orange stain:

Acridine orange dye has an affinity for nucleic acid. When fungi are stained with this dye, RNA component of the cell fluoresces with shades of orange red and DNA component of the cell fluoresces green under fluorescent microscope. Study of Kanungo *et al* in 1994 shows 76% of culture proven fungal isolate demonstrated with acridine orange stain.[43]

FUNGAL CULTURE:

Microbial culture is considered to be the gold standard in the detection of causative organism of corneal ulcer.[56] Inoculated SDA slopes with Gentamicin were incubated aerobically at 25˚c over a period of 6 weeks. Culture was checked every day during first week and twice weekly thereafter. Observe the growth in the plate with 'C' streak [46] and correlate with SDA slope morphology.

Fungal isolates identified by their colony characteristics morphology in obverse and reverse microscopic morphology in lactophenol cotton blue mount [23] and slide culture.[22]

Lactophenol Cotton Blue mount:

Lactophenol Cotton Blue mount was used to observe the hyphal and conidial arrangement and conclude the fungal growth with culture.[93] Thomas *et al* in 1991 and Sharma *et al* in 1998 documented the correlation of macroscopic morphology with microscopic findings in LPCB mount. Kompa *et al* in 1999 used LPCB mount as a sensitive marker in diagnosis.

Slide culture:

The slide culture was performed using isolates. The slide culture is used to study undisturbed morphology details particularly relationship between reproductive structures like conidia, conidiophores and hyphae.[42][95] Fungal slide culture was performed in cases with doubtful morphology.[6]

Adhesive Method for Microscopic Examination of Fungi in Culture were used to improve the identification.[76]

ANTIFUNGAL SUSCEPTIBILITY TESTING:

As resistance patterns to commonly used antifungal drugs continue to shift, sensitivity testing play an important role both in appropriate management of individual cases based on susceptibility characteristics and community surveillance.[61] Standardization of invitro susceptibility testing provides a consistent and reproducible data that predict clinical response when used in conjunction with individual patient risk factors. An ideal

antifungal drug should have a broad spectrum activity, it should be effective in vivo and there should be no drug resistance.

In vitro resistance may be primary and secondary. In primary (intrinsic) resistance occur when the organism was naturally resistant to the antifungal drug. In secondary (acquired) resistance when the isolate producing infection becomes resistant to the antifungal drug during the course of treatment.

Ideally in vitro susceptibility tests were used to,

1. Provide a reliable measure of relative activities of two or more antifungal drugs.

2. Correlate with in vivo activity and predict the likely outcome of therapy.

3. Provide a means with which to monitor the development of resistance among normally susceptible population of organism.

4. Predict the therapeutic potentials of newly discovered investigational agents.

Antifungal susceptibility testing performed by,[57]

(a) Agar based methods:

1. AGAR DILUTION METHOD:[96]

The drug of various concentrations added to the Nutrient agar slope and inoculum suspension was added. The MIC was determined as the lowest concentration of the antifungal drug preventing growth of macroscopically

visible colonies on drug containing plates when there was visible growth on the drug – free control plates.

For MIC determination, the following range of drug concentrations were used,

Amphotericin B : 0.0313-16µg/ml

Itraconazole : 0.0313-16 µg/ml

Fluconazole : 0.125-64 µg/ml

2. DISK DIFFUSION METHOD:

This method is useful in vitro testing of antifungal agent against standard inoculation of fungal pathogen. Disk diffusion method has provide sensitivity pattern of particular fungal pathogen compared with standard zone size. Reference method for disk diffusion susceptibility testing of filamentous fungi , Approved guideline M 51-A followed.[79]

3. E-TEST METHOD:

E- test is a patented commercial method for determination of MIC. In this method calibrated plastic strip impregnated with a concentration gradient of antifungal agent placed over the agar surface and zone of inhibition corresponding to concentration gradient noted. Inoue T *et al* documented E- test in choosing appropriate agents to treat fungal keratitis.[35]

(b) **Broth based methods**:[24]

1. BROTH MACRODILUTION METHOD:

Broth macrodilution was performed in sterile 6 ml polystyrene tubes with a final volume of 1 ml. two times the required concentrations of the drug and the conidial suspension were prepared by two fold serial dilutions.[77]

2. BROTH MICRODILUTION METHOD:

The clinical and laboratory standards institute (CLSI) subcommittee on Antifungal susceptibility tests has been developed a reproducible procedure for antifungal susceptibility testing of filamentous fungi by a broth microdilution format M 38-A2 document for filamentous fungi.[78] It recommends the use of RPMI-1640 medium with glutamine without bicarbonate supplemented with 0.2% glucose and buffered to a pH of 7.0 with 0.165 mol/L MOPS (3-N-morpholinopropane sulfonic acid) .

Inoculum preparation of conidial or sporangiospore suspensions must be adjusted using a spectrophotometer in a range of $0.4x10^4$ to $5x10^4$ CFU/ml to get the most reproducible MIC data. A small drop of Tween 20 as wetting agent added to facilitate the preparation of Aspergillus inoculum.[15] Standard two fold serial dilutions across the concentration range to be tested are made. Good agreement between results obtained by broth microdilution and broth macrodilution methods for moulds has been documented.[124]

3. COLORIMETRIC METHOD:

Tetrazolium salts can penetrate rapidly with intact cells and directly with subcellular membrane with dehydrogenase activity, where they are converted to coloured formazan derivative that can be measured spectrophotometrically at 550nm. Tellier et al in 1992 showed 56% positivity in his study.[97] Pfaller and Barry in 1994 used Alamar blue, anovel colorimetric indicator that changes colour from blue to red.[68]

Other diagnostic methods:

(i) When corneal smears and culture are negative and the keratitis not responding to antifungal therapy, then a diagnostic keratectomy or a corneal biopsy is necessary to establish the diagnosis.

The corneal biopsy specimen should be submitted to the laboratory for smears and cultures. A substantial portion should be submitted for histopathological examination. Histopathological examination of corneal buttons can reveal the presence of fungal elements in 75% of patients.

(ii) Impression cytology and confocal microscopy are other diagnostic tools which are not used routinely. Confocal microscopy is a new and non invasive procedure in which four dimensional view of internal structures are possible at cellular level. Zhonghua *et al* 1999 documented 31 out of 43 patients with fungal keratitis with 96.9% positive rate by confocal microscopy.[102]

(iii) Detection of fungal metabolites by gas liquid chromatography.[36]

(vi) Flow cytometry :

Flow cytometry gives the results within 6 hours. Ramani and Chaturvedi in 2000 reported the antifungal susceptibility of fungal pathogen by flow cytometry.[80]

SEROLOGY:

1. DETECTION OF ANTIBODY:

The antibody production depends on host factor, causative fungus and type of infections. Coleman and kaufman in 1972 found precipitin in 82% of proven cases of fungal corneal ulcer.[119] Solid phase radioimmunoassay has been developed for measurement of antibody which was used in the study by Marier *et al* in 1999.[120] Monoclonal antibody based ELISA was also developed for the detection of antibody levels of fungus causing corneal ulcer.

2. **DETECTION OF ANTIGENS:**

The serological test for detection of antigens are of limited value in early stages of infection, in patient with impaired immunity or immune response is not sufficient to raise significant level of antibodies. Latex particle agglutination test for detection of antigen were used. Radioimmunoassay (RIA) shows 70-80% sensitivity in study conducted by Talbot *et al* in 1987.[121] Sabetta *et al* in 1985 demonstrate antigen by

competitive enzyme immune assay (EIA) in five of six immunocompromised cases with invasive fungal infection.[122]

MOLECULAR DIAGNOSIS:

Polymerase chain reaction:

Polymerase chain reaction amplification can be used to detect the presence of as few as 10 organisms per 100ml volume of clinical specimen.PCR used to detect segment of fungus specific DNA coding for cytochrome $P450L_1A_{1,}$ chitin synthase gene, 18S RNA gene.

Thomas G. Mitchell *et al* in 2002 documented the pathogenic fungi by Multiplex PCR directly from the cultures.

Detection of fungi in scrapings from infected corneas by polymerase chain reaction (PCR) based assay to amplify a portion of the fungal 18S ribosome gene in the study of P A Gaudio *et al in* 2002.[31]

Corneal scrapings are processed for DNA extraction which is amplified by fungal specific primers of internal transcribed spacer region 1 (ITS 1). The products are sequenced and analysed by single standard conformation polymorphism (SSCP) for species identification.[55]

Manish kumar *et al* in 2005 has reported, the sensitive and rapid polymerase chain reaction based diagnosis of mycotic keratitis through single standard conformation polymorphism in their study. [55]

Detection and Identification of fungal pathogen by PCR and by ITC2 and ribosomal DNA typing in ocular infection by Consuelo Ferrer *et al* in 2001.

P.A .Gaudio *et al* in 2002 concluded that PCR is promising as a means to diagnose fungal keratitis and offers some advantages over culture methods, including rapid analysis and the ability to analyse specimens.

Recently, Novel Real time PCR assays targeting the fungal ITS2 (internal transcribed spacer region 2) are developed for the detection and differentiation of medically important Aspergillus species and Candida species using light cycler instrument.[13]

STEM CELL THERAPY:

Stem cell therapy is upcoming therapeutic modality in recent years in medical field. A study conducted by department of cornea, Vision research foundation, Chennai in animal model (Rabbit) shows that transplantation of autologous limbal epithelial cells grown in thermo reversible polymer Mebiol Gel may restore a nearly normal ocular epithelial surface in eyes.[89]

Another study by Vision research foundation, Chennai collaborate with Nichi-in centre for regenerative medicine, Chennai in 2009 reported using a scaffold, for example Human amniotic membrane, collagen, polymer to restore sight in the damaged eye by transplanting the limbal stem cells from the healthy eye to the damaged eye.[69]

TREATMENT OF CORNEAL ULCER:

Natamycin 5% suspension, Amphotericin B is used routinely in the treatment of corneal ulcer. The Azoles and Flucytocin are generally used an alternative agents in advanced ulcers.[63]

Mohan *et al* in 1989, obtained success rate of 64.7%, when 1% Miconazole was used to treat smear positive keratitis.[59]

Oral Fluconazole and Itraconazole have good intraocular penetration with few adverse effects compared to other azoles.[21]

Newer agents like as triazoles (Posaconazole, Ravuconazole), Echinocandins, Sodarin derivaties and the Nikkomycins will improve the treatment of fungal corneal ulcer.[26]

Surgical treatment of corneal ulcer:

Frequent cornea debridement with a spatula is helpful which debulks fungal organisms and epithelium and enhances penetration of the topical antifungal agents.[21]

Although mainstay of initial management of severe keratitis remains aggressive antimicrobial therapy, the role of timely surgical intervention in the form of therapeutic keratoplasty[117] should be considered in patients with severe end stage diseases. The timing of surgery was critical. The surgery should be performed within 4 weeks of presentation. Therapeutic keratoplasty may effectively treat severe refractory infectious corneal ulcers.[87]

Aim and Objectives

AIM AND OBJECTIVE OF THE STUDY

- To find out the spectrum of fungal pathogens causing corneal ulcers in the patients attending a tertiary care hospital in Chennai.
- To try and establish the etiopathogenesis of these infections.
- To identify the predisposing factors for fungal corneal ulcers.
- To evaluate the efficacy of diagnostic methods for isolation of corneal pathogens.
- To study the sensitivity pattern of fungal isolates to the commonly used antifungal drugs.

Materials and Methods

MATERIALS AND METHODS

The study was conducted to know the spectrum and etiopathogenesis of fungal organism causing keratitis and to evaluate by culture techniques in isolation of fungus causing corneal ulcer.

The study group comprised of 160 patients attending the cornea clinic at Govt. Regional Institute of Ophthalmic Hospital, Chennai during the period from June 2009 to May 2010.

INCLUSION CRITERIA:

(b) Patients having proven corneal ulcer on clinical examination, attending cornea clinic.

(c) Both outpatient and inpatient were included in the study.

(d) Patients under treatment for corneal ulcer with follow up.

(e) Postoperative patients of ocular surgery with suspicion of impending corneal ulcer.

COLLECTION OF SPECIMENS:

Written consent from the participants (or) their guardians included in of the study was obtained after providing full explanation of the current study in their local language. The study was submitted to Institutional Ethical committee and got the approval for proceedings. All the data collected were kept confidential.

Specimens were taken from patients of corneal ulcer and follow-up patients with corneal ulcer. Informed consent was obtained from the patients and data were collected as per proforma. Corneal scrapings were collected for investigations.

1. Patient was made to lie down comfortably on a couch.

2. The affected eye was cleaned with sterile saline using sterile swabs.

3. Sterile 2% Xylocaine was applied to the eye taking care not to apply too much of it as it may inhibit the growth of the organism.

4. Care was taken to see that the eyelids did not contaminate the specimens. Eye speculum was used whenever necessary.

5. Patients were given relevant instructions regarding position and restriction of eyeball movement during the scraping procedure.

6. No.15 Bard Parker blades were used to scrap the ulcer. A new sterile blade was used for each patient.

7. Materials were obtained from leading edge and base of each ulcer Scrapings were taken and processed as follows,

(a) Specimen was applied to two sterile microscope slide for 10% KOH mount and Gram stain.

(b) Specimens were inoculated into two Sabouraud's dextrose agar slants with antibiotics (Gentamicin) without Cycloheximide.

(c) Specimen was streaked in a "C" shaped manner in a Sabouraud's dextrose agar plate.

SPECIMEN PROCESSING:

1. **10% POTASSIUM HYDROXIDE MOUNT:**

The scraping material was transferred onto a clean glass slide and one or two drops of sterile 10% KOH was applied over that and covered with clean coverslip without introducing air bubbles and examined under low and high power objective for the presence of hyphal elements , conidial forms of the fungal isolates. KOH digests proteinacious material and retain the polysaccharide fungal cell wall. The results will be correlated with culture report later.

2. **DIRECT SMEAR**:

The corneal scraping material was transferred onto a clean glass slide with a drop of sterile normal saline. The smear was made using a sterile bacteriological loop. The smear was allowed to air dry and heat fixed. The prepared smear was stained by Gram stain method and examined under oil immersion objective and observed for presence of polymorphs, mononuclear cells, epithelial cells, bacteria (Gram positive & Gram negative), yeast like cell, if present their nature and relative number were noted. Bacterial pathogen identified and processed.

3. **CULTURE METHOD:**

Microbial culture is considered to be the gold standard in the detection of causative organism of corneal ulcers. The Bard Parker blade containing the scraping material was slightly depressed in to the medium, to that the

specimen was left on the surface. Then it was streaked with a sterile wire loop and incubated aerobically at 25˚c.

Microbial culture were considered significant,

(a) If growth of same fungal species observed in more than one culture slope (or) plate.

(b) If there was confluent growth at the site of inoculation in solid media.

(c) Growth was consistent with microscopic findings (KOH mount, Gram stain).

(d) If the same organism was grown from repeated scraping from the patients.

Fungal isolates were identified by studying the colony morphology on the Sabouraud's dextrose agar slope, colony colour, production and arrangement of conidia in preparation stained by Lactophenol cotton blue mount.

When identification was difficult due to inadequate sporulation, Riddles slide culture technique was employed. In the case of yeast identification and speciation was done by Gram stain morphology, Germ tube test, morphology on Corn-meal agar and biochemical test by standard microbiological techniques.

EXAMINATION OF INOCULATED MEDIA:

The colonies were observed for growth in the Sabouraud's dextrose agar slopes and noted the description, if it was inadequate reincubated. The Sabouraud's dextrose agar slopes were examined daily during first week and twice a week for next 3 weeks. Failure of growth after 6 weeks was considered as negative for fungal growth and is to be discarded.

LACTOPHENOL COTTON BLUE STAIN :

The fungal growth was taken from Sabouraud's dextrose agar slope with spud and transferred onto the clean glass slide and two to three drops of Lactophenol cotton blue reagent was added over the fungal growth. By using teasing needles the growth was spread over the slide and coverslip was placed without trapping any air bubbles. The morphology of hyphae, conidia were observed under microscope and was correlated with macroscopic features.

RIDDLE'S SLIDE CULTURE METHOD:

This was used to study the undisturbed morphological details of fungi, particularly relationship between reproductive structures like conidia conidiophores and hyphae. Fungal slide culture was performed in cases with doubtful morphology.

1. A round piece of filter paper was placed on the bottom of a sterile Petri dish. A pair of thin glass rods was placed on top of the filter paper to serve as supports 3 inch x 1 inch glass microscopic slide. 3 to 4 coverslips were placed within the petridish and sterilized as a whole.

2. 1x1 cm square block of Sabouraud's dextrose agar was cut from a petridish by using sterile scalpel and transferred the agar block to the microscope slide.

3. Four sides of the agar block were inoculated with a fungal colony to be studied by using heavy gauge nichrome wire.

4. The agar block was covered with sterile coverslip in the petridish.

5. Moisten the filter paper with sterile water and place the lid on the petridish.

6. The Petridish was incubated at room temperature and examined periodically for growth.

7. When a growth visually appeared to mature, the coverslip was gently lifted from the surface of the agar with a pair of forceps taking care not to disturb the mycelium adhering to the bottom of the coverslip.

8. The coverslip was placed on a small drop of Lactophenol cotton blue on a second glass slide. Likewise, the mycelium adhering to the surface of the original glass slide after the block removed also was stained with Lactophenol cotton blue and a fresh coverslip was overlaid.

9. The characteristic shape and arrangement of hyphae, conidia were observed microscopically.

The mycelia which adhere to the glass surface usually show characteristic microscopic appearance which may be lost if needles are used to tease as it happens in the routine Lactophenol cotton blue mounts. The slide culture may also be seen directly by placing under low power of the microscope.

The cellophane tape preparation has come into greater use to overcome the obstacles of time consumption and requirement of the extra equipment to prepare the slide culture. A piece of tape is gently laid over a portion of the fungal colony and slowly lifted to remove an area of the colony and placed on a microscope slide with a drop of Lactophenol cotton blue and examined under low power of the microscope. This preparation becomes an instant slide culture, revealing relationship of the various fungal structures.

GERM TUBE TEST:

If the Sabouraud's dextrose agar slope shows cream coloured, smooth and pasty colonies after 3-4 days of incubation. The culture of Candida species was inoculated into 0.5 ml of mammalian serum from fetal bovine, sheep or normal human beings and incubated at 37˚c for 2-4 hours. A drop of suspension was taken on slide and examined under the microscope. The germ tubes are seen as long tube-like projections extending from the yeast cells.

They grow at the distal end. There was no constriction at the point of attachment to yeast cell. The germ tubes are formed within two hours of incubation in Candida albicans. The preparation should not be incubated longer than 4 hours because other hyphae producing yeasts might begin to germinate beyond this time.[123]

INVITRO SUSCEPTIBILITY TESTING:

The National Committee for Clinical laboratory Standards (NCCLS) which describes the standard parameters for testing MIC (Minimum Inhibitory Concentration) of established agents against filamentous fungi.

Antifungal susceptibility testing is receiving attention with the advent of newer anti fungal drugs. However susceptibility testing of filamentous fungi is not as advised as susceptibility testing. In vitro susceptibility tests should provide a reliable measure of relative activity of the antifungal agent, correlate with in vivo activity and predict the likely outcome of the therapy, provide a means with which to monitor the development of resistance and predict the therapeutic potentials of newer drugs.

Invitro Susceptibility Testing of fungi is influenced by a number of technical variables such as inoculums size and preparation, medium composition and pH, duration and temperature of incubation and MIC end point determination. In addition there are problems unique to fungi like their slow growth rates and the ability of some of them to grow either as yeasts

with blastoconidia or as moulds with variety of conidia depending on pH, temperature and medium composition.

DISK DIFFUSION METHOD:

1.Inoculum preparation:

The fungal colony to be tested was grown in Potato dextrose agar slants at 35˚c to induce the conidium and sporangiospore formation. After 7 to 10 days of incubation with well grown spores, the culture was taken for testing.

1. 5 ml of 0.85% sterile saline was added to the culture tube and the suspension were made by gently probing the colonies with the tip of Pasteur pipette.
2. With the help of sterile pipettes, the saline with conidia was transferred into a sterile screw cap tube.
3. The tube was then vortexed for 30 seconds to one minute. The tube was allowed to stand at room temperature for 5 to 10 minutes for the heavier particles to settle down.
4. The upper homogenous suspensions were collected and the densities of the conidial suspensions were read and adjusted to an optical density (OD) that ranged from 0.09 to 0.11 for Aspergillus species, 0.15 to 0.17 for Fusarium species by using spectrophotometer at 530 nm

65-70% absorbance. A small drop of Tween 20 as wetting agent added to facilitate the preparation of Aspergillus inoculums.

5. These suspensions were diluted 1:50 in RPMI 1640 medium.
6. The final concentration of the conidia should be 0.2 - 1 X 10^4 cfu/ml.
7. The inoculum preparation procedure was same for Agar dilution and Broth dilution methods.

2. Medium:

Disk diffusion test was performed on Muller-Hinton agar plates supplemented with 2% glucose and 0.5 µg/ml Methylene blue.

3. Procedure:

The entire dried agar surface was evenly streaked in three different directions with a sterile cotton swab dipped into the inoculum suspension. The plate was allowed to dry for 10 minutes. Using a pair of flame sterilized forceps the antifungal disks were applied onto the surface of the inoculated plate. The plates were incubated at 35˚c for 48 hours. The plates were read at 24 hrs and 48 hrs.

The following commercial Hi-Media antifungal disks were used.

Amphotericin B 20 µg	Itraconazole 10 µg
Fluconazole 25 µg	Voriconazole 1 µg

The following standard strains were tested each time to ensure quality control. Aspergillus flavus ATCC 204304

Aspergillus fumigatus ATCC 204305

4. Interpretation:

Zone diameters were measured to the nearest whole millimeter at the point where there was prominent reduction of growth. The results were compared with Broth microdilution method for respective fungal isolates.

AGAR DILUTION METHOD:

1. Medium:

Agar dilution method was performed on Nutrient agar or Muller-Hinton agar plates supplemented with 2% glucose and 0.5 µg/L Methylene blue.

2. Procedure & interpretation:

1. 1.8 ml of molten Nutrient agar poured into sterile test tubes and allowed to cool to 50˚c.
2. 0.2 ml of drug dilutions from stock solution added in descending concentration to NA slope.
3. 100 µl of standardized inoculums added to all tubes except sterility control tube.
4. Tubes incubated at 30˚c for 2 days.
5. Visualized macroscopically for growth.
6. Lowest concentration of the drug which permitted no macroscopically visible growth after 2-3 days is taken as MIC.

BROTH MICRODILUTION METHOD

1.Growth Medium Preparation:

1. The completely synthetic medium Rosewell Park Memorial Institute – 1640 (RPMI-1640) supplemented with 0.3g of L-glutamate per liter without sodium bicarbonate was used as a growth medium in antifungal susceptibility testing. The medium should be buffered at the pH of 7.0 ± 0.1 at 35˚c.
2. The buffer used was MOPS (3-N-morpholinopropane sulfonic acid) with final concentration of 0.165 mol/L with ph of 7.0 .
3. RPMI 1640 was dissolved in MOPS. The final solution was sterilized by filtration through membrane filter and stored at 4˚c.
4. The same medium was used for the preparation of the drug dilutions.

2.Drug Dilution Preparation:

1. The drug dilutions were prepared following the additive twofold drug dilution scheme described in the NCCLS M38-A method.
2. Stock drug solutions were first diluted to 100x the final concentration in 100% dimethyl sulfoxide (DMSO) and further diluted 1:50 in 2x medium to obtain the 2x drug concentration. The final drug concentration was 0.125 to 32µg/ml for Amphotericin B and 0.0313 to 16 µg/ml for Itraconazole. Fluconazole was dissolved in sterile distilled water and final drug concentration was made from 2 to 256 µg/ml.

3. These volumes were adjusted according to the total number of tests required. Because there will be 1:2 dilution of the drug when combined with the inoculum, working antifungal solutions were 2 fold more concentrated than the final concentration.

3.Inoculation In RPMI – 1640 Medium:

1. The inoculation was done in sterile 96 - well microtitre plate with flat bottom.
2. Each well was inoculated with 100 µl of the conidial suspension.
3. 100µl of the diluted drugs were added correspondingly to each well.
4. The growth control well was inoculated only with the 200 µl of diluted conidial suspension with the growth medium without any antifungal agents.
5. The sterility control well was inoculated with 200 µl of the growth medium alone without any conidium.
6. All microtitre plates were incubated at 35°C for 48 hours without agitation and evaluation was done after four days of incubation.

4.Reading MIC :

1. The test was read when the growth control shows adequate growth, which is typically 24-48 hours for most moulds, but it could be up to 96 hours.
2. Read MICs the first day that the growths controls showed the visible growth and then 24 hours later.

3. Scores were given as follows,

   (1) 0 = optically clear

   (2) 1+ = slightly hazy

   (3) 2+ = prominent reduction in turbidity compared with that of the drug-free growth control

   (4) 3+ = slight reduction in turbidity compared with that of the drug-free growth control

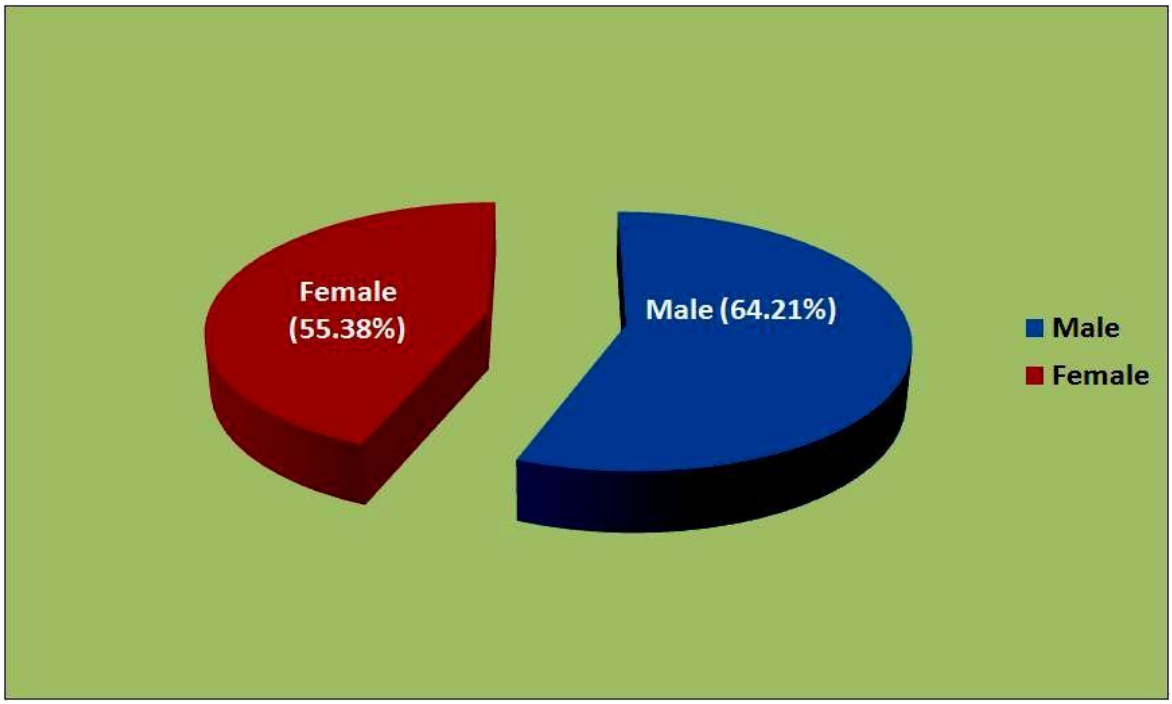
   (5) 4+ = no reduction in turbidity compared with that of the drug-free growth control.

STATISTICAL ANALYSIS:

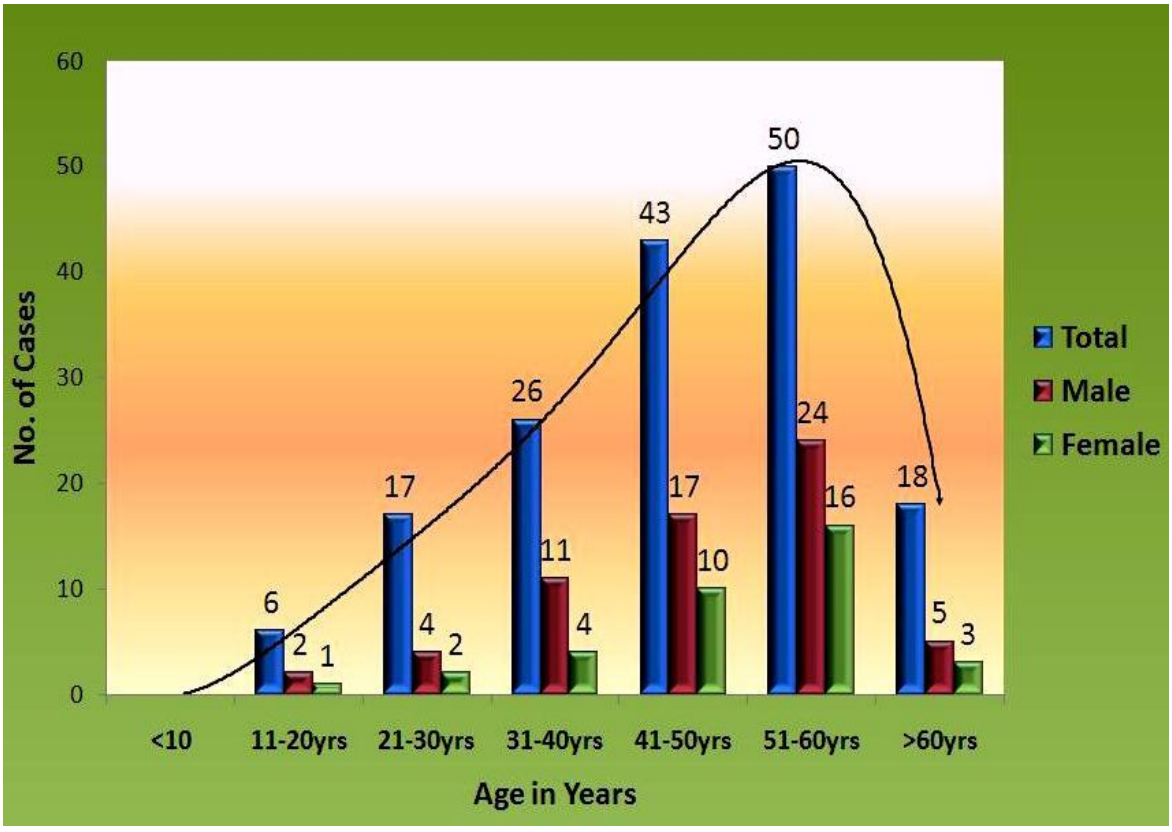
A statistical analysis was carried out using statistical package for social sciences (SPSS) and Epi-info software by a statistician. The proportional data of the cross sectional study was tested using Pearson's Chi-square analysis test and Binomial proportion test.

Results

GENDER DISTRIBUTION OF INFECTIOUS CORNEAL ULCER

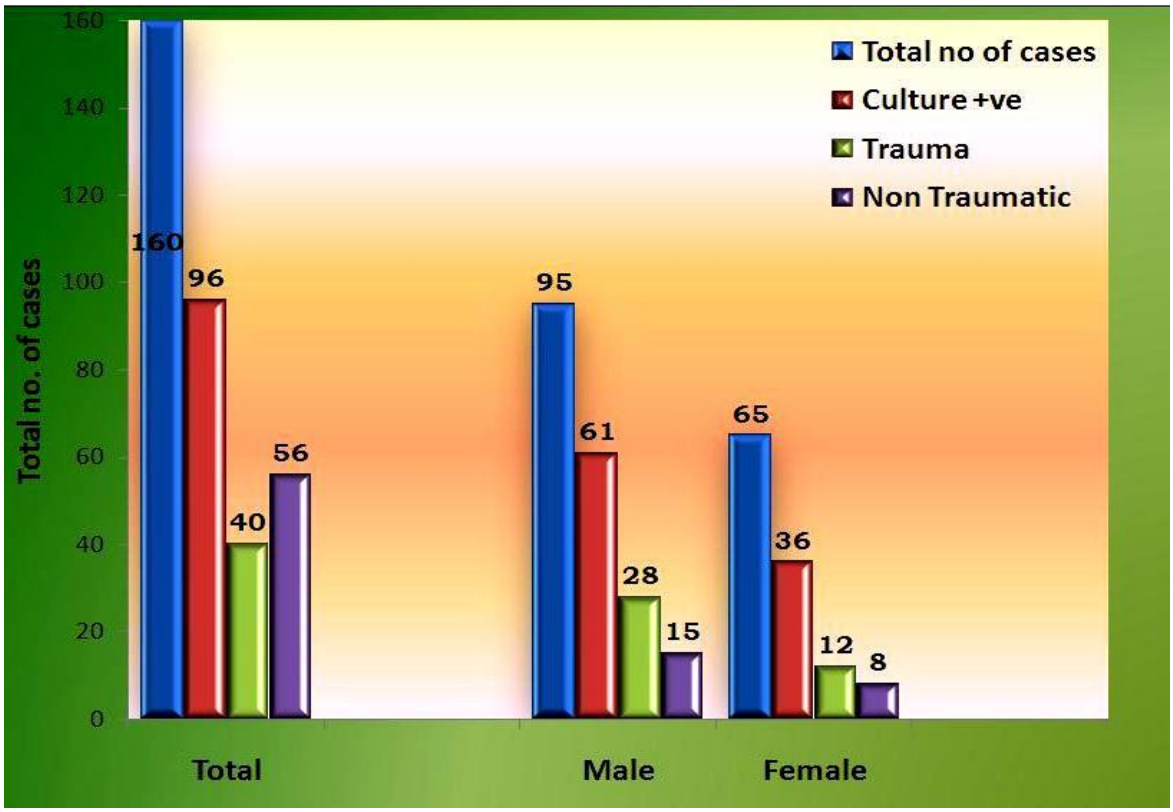


AGE DISTRIBUTION OF INFECTIOUS CORNEAL ULCER

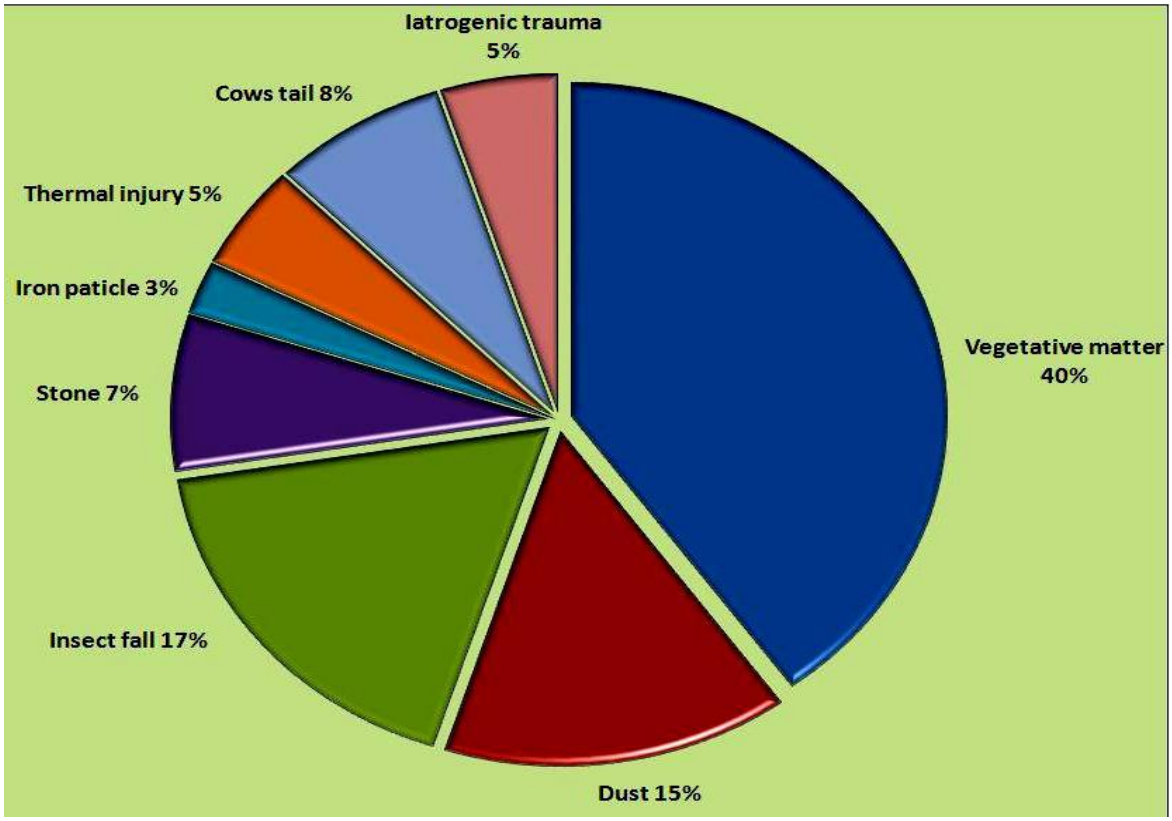


Prevalence of corneal ulceration was more common in 51-60 yrs of age group.

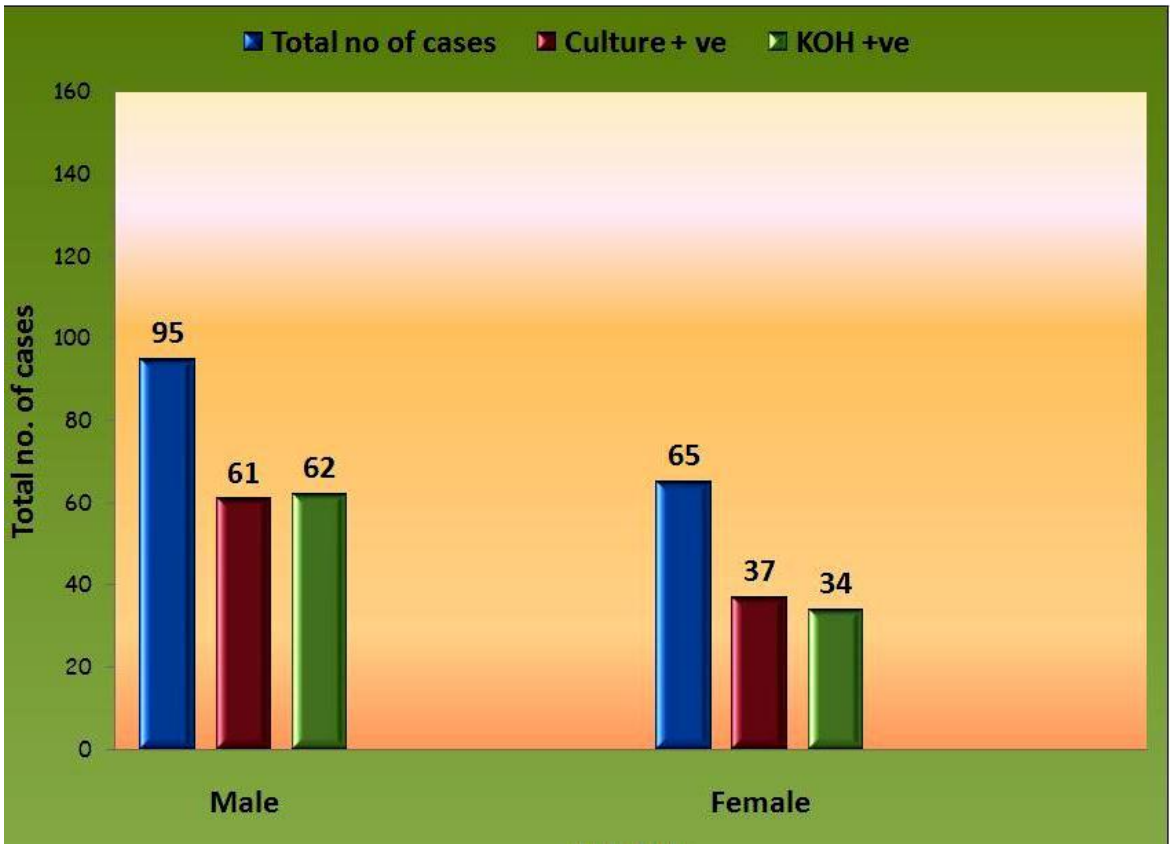
DISTRIBUTION OF PREDISPOSING FACTORS IN CORNEAL ULCER



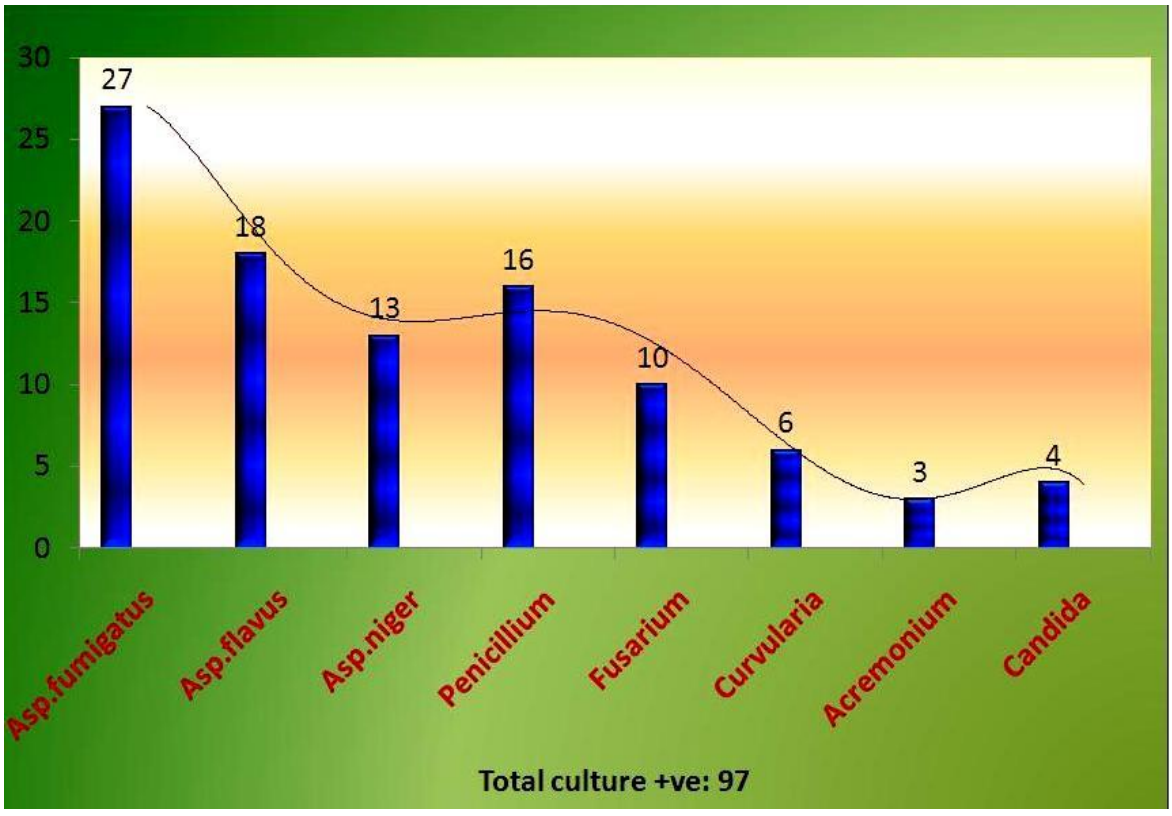
DISTRIBUTION OF TRAUMA FACTORS IN CORNEAL ULCER



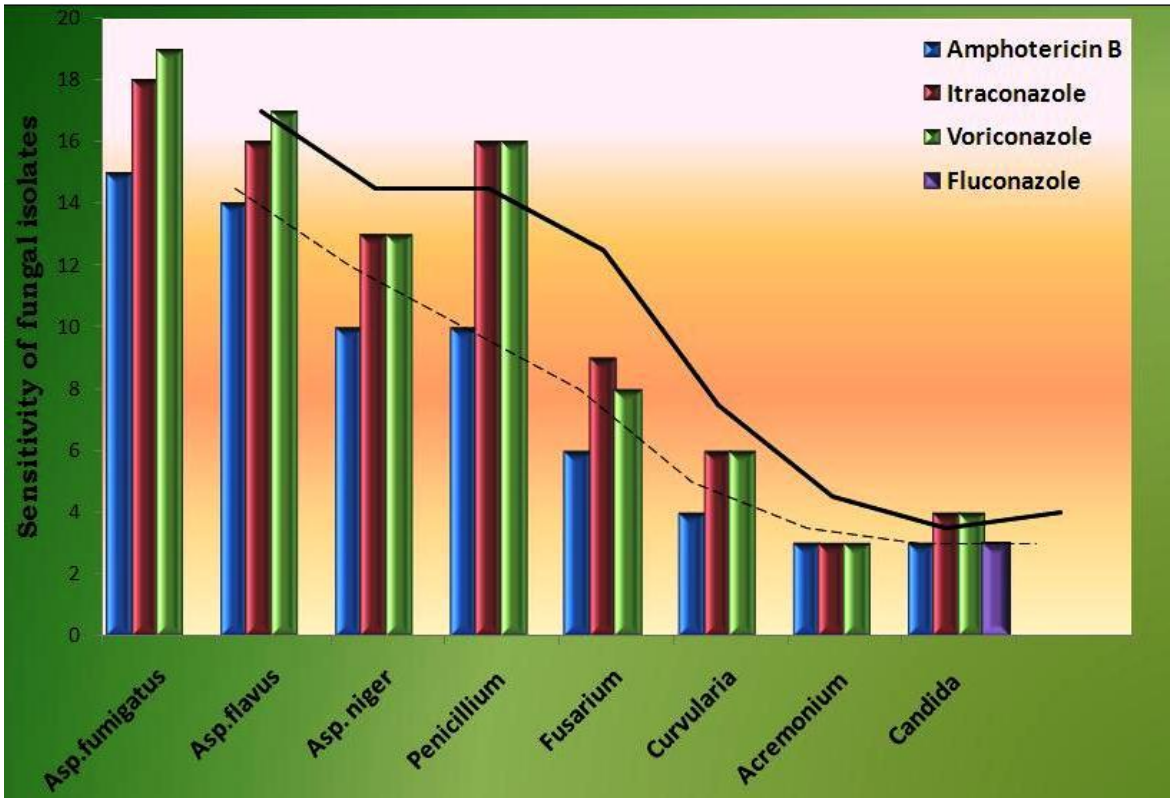
GENDER DISTRIBUTION WITH CULTURE POSITIVITY AND 10% KOH MOUNT



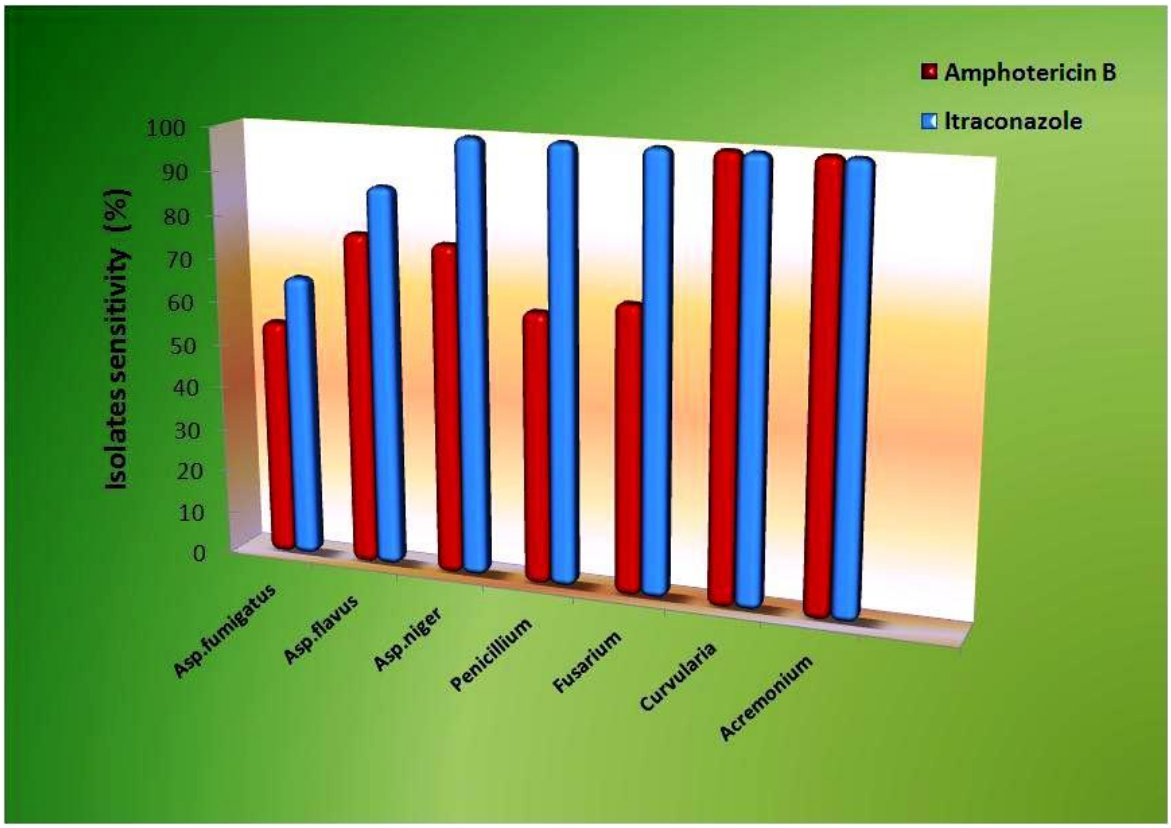
DISTRIBUTION OF FUNGAL AGENTS CAUSING CORNEAL ULCER



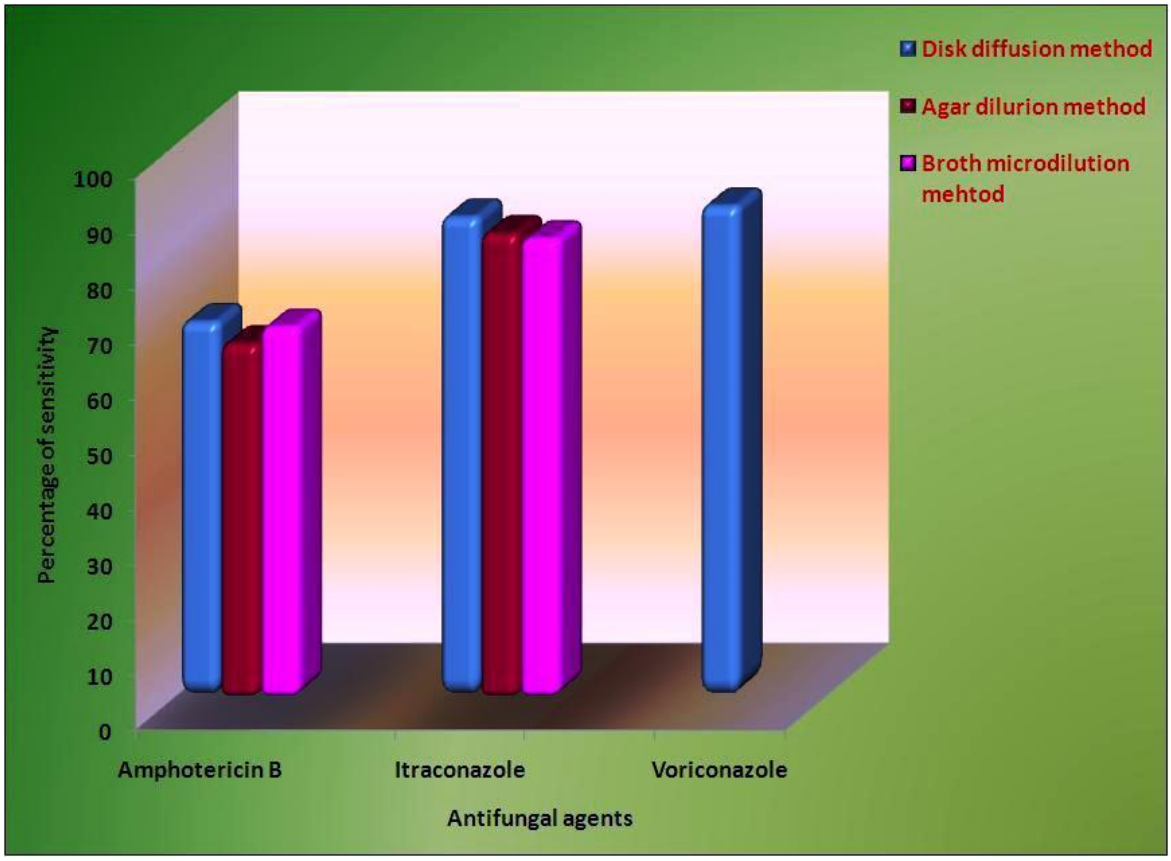
ANTIFUNGAL SUSCEPTI BILITY TESTING DISK DIFFUSION METHOD



MINIMUM INHIBITORY CONCENTRATION AGAR DILUTION METHOD



ANTIFUNGAL SUSCEPTI BILITY TESTING
SENSITIVITY OF ANTIFUNGAL AGENTS



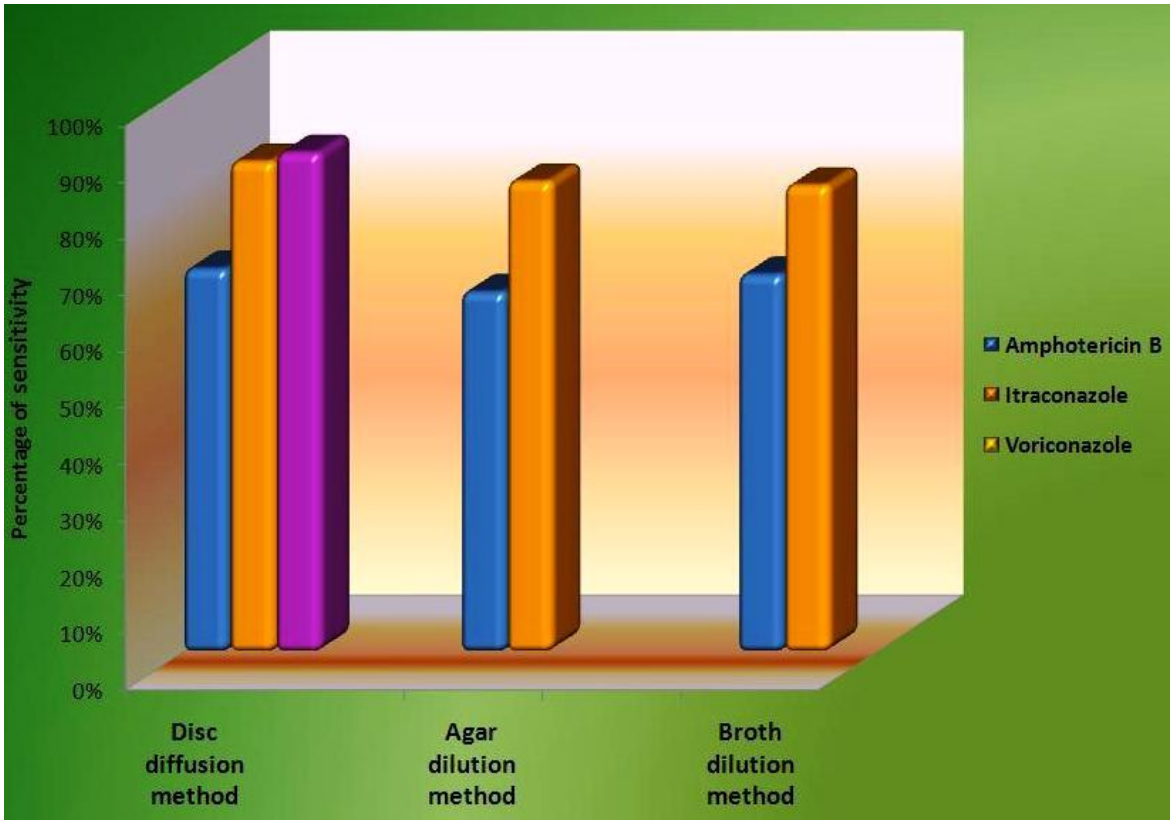


TABLE OF RESULTS

TABLE 1

CULTURE POSITIVITY IN THE CORNEAL SCRAPING SAMPLES N=160

Total no. samples collected	No. of Culture positive samples	Percentage of culture positivity
160	97	60.6%

TABLE 2

GENDER DISTRIBUTION OF INFECTIOUS CORNEAL ULCER N=160

Gender	Total No. of cases	No. of culture positives	Percentage
Male	95	61	64.21%
Female	65	36	55.38%

Male gender have increased incidence of keratitis probably because of their occupation. P = 0.005 significant.

TABLE 3

AGE DISTRIBUTION OF INFECTIOUS CORNEAL ULCER N=160

Age (Years)	Total No. of cases	No.of culture positive		Percentage of cases on total culture positive (%)
		Males	Females	
10	-	-	-	-
11-20	6	2	1	3.0
21-30	17	4	2	6.1
31-40	26	10	4	14.4
41-50	43	16	10	26.8
51-60	50	24	16	41.2
>60	18	5	3	8.2
Total	160	61	36	100.0

Prevalence of corneal ulceration was more common in 51-60 yrs of age group.

TABLE 4

DISTRIBUTION OF PREDISPOSING FACTORS CAUSING CORNEAL ULCER

Gender	Total No. of cases	Culture positive	Traumatic origin	Percentage (%)
Male	95	61	28	45.9
Female	65	36	12	33.3

TABLE 5

DISTRIBUTION OF CORNEAL ULCER AMONG TRAUMATIC CASES

Nature of trauma	Male	Female	Total	Percentage (%)
Vegetative matter	11	5	16	40
Dust	4	2	6	15
Insect bite	5	2	7	17.5
Stone	2	1	3	7.5
Iron particle	1	-	1	2.5
Thermal injury	2	-	2	5
Cows tail	2	1	3	7.5
Iatrogenic trauma	1	1	2	5
Total	28	12	40	100

Among traumatic cases, vegetative matter and insect bite comprise more than half of the corneal ulcer cases. P=0.001 significant.

TABLE 6

DISTRIBUTION OF PREDISPOSING FACTORS OTHER THAN TRAUMA

Non traumatic origin	Male	Female	Percentage (%)
H/O Steroid intake (topical & inhalational)	4	2	10.5
H/O prior antifungal use Follow up cases	7	3	17.54
Postoperative (cataract, keratoplasty)	2	1	5.2
Leprosy	1	-	1.7
Bell's palsy	1	-	1.7
Native medicine installation	-	2	3.5

Steroid induced corneal ulceration show high prevalence among nontraumatic cases.

Inappropriate dose and duration of antifungal usage can form a base for resistance strains.

TABLE 7

DISTRIBUTION OF FUNGAL AGENTS CAUSING CORNEAL ULCER

Fungal Agent	Total isolates	No. of isolates		Percentage (%)
		Male	Female	
Aspergillus fumigatus	27	18	9	27.83
Aspergillus flavus	18	11	7	18.55
Penicillium species	16	9	7	16.49
Aspergillus niger	13	8	5	13.40
Fusarium species	10	7	3	10.30
Curvularia species	6	3	3	6.18
Acremonium species	3	2	1	3.09
Candida albicans	4	3	1	4.12

Aspergillus species were the most common fungal agent isolated (59.7%).

TABLE 8

SMEAR POSITIVITY AMONG CORNEAL ISOLATES

Gender	Total No. of specimens	10%KOH positivity	Gram stain positivity (yeast like cells)
Male	95	62	2
Female	65	34	-

TABLE 9

10% POTASSIUM HYDROXIDE SCREENING TEST

10 % KOH Mount	Culture		Total
	Positive	Negative	
Positive	94	2	96
Negative	3	61	64
Total	97	63	160

Sensitivity : TP/(TP+FN) = 96.9%

Specificity : TN/(TN+FP) = 96.8%

TABLE 10

ANTIFUNGAL SUSCEPTI BILITY TESTING
DISK DIFFUSION METHOD

Organism	No. of isolates	Ampho B (20µg) S>15mm	Itraconazole (10µg) S>23mm	Voriconazole (1µg) S>17mm
Aspergillus fumigatus	27	15 (55%)	18 (66%)	19(70%)
Aspergillus flavus	18	14 (77%)	16 (88%)	17 (94%)
Aspergillus niger	13	10 (76%)	13 (100%)	13 (100%)
Penicillium species	16	10(62.5%)	16 (100%)	16 (100%)
Fusarium species	10	6 (60%)	9 (90%)	8 (80%)
Curvularia species	6	4 (66%)	6 (100%)	6 (100%)
Acremonium species	3	3 (100%)	3 (100%)	3 (100%)
Candida albicans	4	3 (75%)	4 (100%)	4 (100%)

75% (3/4) of Candida albicans were sensitive to Fluconazole (Fu 25µg) with zone size >19mm. Other fungal isolates were resistant (<15mm) to Fluconazole.

Aspergillus niger and Acremonium species were 100% sensitive to azoles group of drugs.

TABLE 11

MINIMUM INHIBITORY CONCENTRATION AMPOTERICIN B AGAR DILUTION METHOD

Organism	0.25µg	0.5µg	1µg	2µg	4µg	8µg	16µg	32µg	64µg
Asp.fumigatus	2	4	7	2	4	3	1	3	1
Asp. flavus	3	4	4	3	2	1	1	-	-
Asp. niger	2	5	2	1	2	1	-	-	-
Penicillium sp.	-	4	2	4	3	2	1	-	-
Fusarium sp.	-	1	3	2	3	-	1	-	-
Curvularia sp.	-	1	1	2	2	-	-	-	-
Acremonium sp.	-	2	1	-	-	-	-	-	-

39/58 (67.2%) Aspergillus species, 62.5 % (10/16) of Penicillium isolates, 66 % (4/6) of Curvularia species and 100% of Acremonium species showed sensitive range for Amphotericin B. (MIC of less than 2µg/ml)

TABLE 12

MINIMUM INHIBITORY CONCENTRATION ITRACONAZOLE AGAR DILUTION METHOD

Organism	0.125µg	0.25µg	0.5µg	1µg	2µg	4µg	8µg	16µg
Asp.fumigatus	-	4	4	7	3	4	3	2
Asp. flavus	2	3	5	4	2	2	-	-
Asp. niger	2	4	4	2	1	-	-	-
Penicillium sp.	1	3	6	5	1	-	-	-
Fusarium sp.	-	2	4	2	1	1	-	-
Curvularia sp.	1	2	3	-	-	-	-	-
Acremonium sp.	-	-	3	-	-	-	-	-

Itraconazole was found to be more effective with MIC<2 µg against the fungal isolates.

TABLE 13

MINIMUM INHIBITORY CONCENTRATION AMPOTERICIN B BROTH MICRODILUTION METHOD

Organism	0.25µg	0.5µg	1µg	2µg	4µg	8µg	16µg	32µg	64µg
Asp. fumigatus	2	3	6	3	5	3	3	2	-
Asp. flavus	3	4	3	4	2	2	-	-	-
Asp. niger	4	3	3	1	1	1	-	-	-
Penicillium sp.	2	2	4	3	3	2	-	-	-
Fusarium sp.	1	1	3	3	1	1	-	-	-
Curvularia sp.	1	2	-	1	2	-	-	-	-
Acremonium sp.	-	2	1	-	-	-	-	-	-

TABLE 14

MINIMUM INHIBITORY CONCENTRATION ITRACONAZOLE BROTH MICRODILUTION METHOD

Organism	0.125 µg	0.25µg	0.5µg	1µg	2µg	4µg	8µg	16µg	32µg
Asp. fumigatus	-	5	4	6	3	4	4	1	-
Asp. flavus	1	3	5	4	3	1	1	-	-
Asp. niger	2	5	5	1	-	-	-	-	-
Penicillium sp.	2	4	4	5	1	-	-	-	-
Fusarium sp.	1	2	3	1	2	2	-	-	-
Curvularia sp.	1	2	2	1	-	-	-	-	-
Acremonium sp.	-	2	1	-	-	-	-	-	-

TABLE 15

COMPARISION OF MIC IN AGAR DILUTION AND BROTH MICRODILUTION

Drug concentration	Amphotericin B MIC<2µg		Itraconazole MIC <2µg	
Organism	Agar Dilution Method	Broth microdilution Method	Agar Dilution Method	Broth microdilution Method
Aspergillus fumigatus	15	14	18	18
Aspergillus flavus	14	14	16	16
Aspergillus niger	10	11	13	13
Penicillium species	10	11	16	16
Fusarium species	6	8	9	8
Curvularia species	4	4	6	6
Acremonium species	3	3	3	3
Percentage	63.9%	67%	83.5%	82.47%

A good correlation were observed between Agar dilution method and Broth microdilution method in the sensitivity pattern of fungal isolates with antifungal drugs.

TABLE 16

MINIMUM INHIBITORY CONCENTRATION AMPOTERICIN B BROTH MICRODILUTION METHOD

Organism	MIC range	MIC_{50}	MIC_{90}
Asp. fumigatus	0.25-64	2	16
Asp. flavus	0.125-32	1	4
Asp. niger	0.125-16	0.5	2
Penicillium sp.	0.125-16	1	4
Fusarium sp.	0.25-64	1	4
Curvularia sp.	0.25-19	0.5	4
Acremonium sp.	0.125-8	0.5	2

TABLE 17

MINIMUM INHIBITORY CONCENTRATION ITRACONAZOLE BROTH MICRODILUTION METHOD

Organism	MIC range	MIC_{50}	MIC_{90}
Asp. fumigatus	0.25-64	1	8
Asp. flavus	0.0625-16	0.5	2
Asp. niger	0.0313-8	0.25	0.5
Penicillium sp.	0.0625-4	0.25	1
Fusarium sp.	0.0625-8	0.5	4
Curvularia sp.	0.0313-4	0.25	1
Acremonium sp.	0.125-2	0.25	1

RESULTS

A total of 160 patients with infectious corneal ulcer were selected for the study. 97 cases were culture positive (60.6%). (Table 1)

The cases were analyzed under the following parameters.

The age and sex distribution of infectious corneal ulcer was analyzed. 95 males and 65 females among these patients were studied.(Table 2).

P = 0.005 significant. 88.7% (142/160) cases were found to be in age group between 10-60 years and 31.25% (50/160) of cases were in the age group of 51-60 years. Extremes of the age group showed low prevalence of corneal ulceration. (Table 3).

Considering the sex distribution 61(64.21%) males and 36 (55.38%) female patients showed positive culture. A high prevalence of fungal corneal ulcers was seen among males contributing to 64.21% of cases. (Table 2)

The age and sex distribution of the patients along with the positive culture for fungi were shown in Table 2&3. From this it seems that the maximum incidence of infected fungal corneal ulceration was in the fifth decade.

The urban and rural distribution of cases showed higher prevalence of fungal corneal ulcer in rural population accounting for 68.2%.Numerous predisposing factors have been implicated in the development of infectious

corneal ulcer of which trauma alone contributed to 41.23% of the cases. (Table 4&5) .

Steroid administration, post ocular surgery, native medicine installation account for 23.71% cases among nontraumatic origin of infectious fungal corneal ulcer. (Table 6). The relationships of influence of the various predisposing factors on the isolation of corneal pathogens were shown in Table 5&6.

In analyzing the contribution of different trauma lesions in fungal corneal ulcer, trauma with vegetative matter like paddy, leaf, wood and were implicated in 37.5% of cases. (Table 5).

The present study was carried out during the period of June 2009 to May 2010.The incidence of corneal ulceration due to fungus were more often during the hot , dry , windy months .

Among the fungal isolates 58 out of 97 (59.7%) cases were due to Aspergillus species, and next common agent isolated was Penicillium species (16.49%) followed by Fusarium species (10.3%), Curvularia species (6.18%), Acremonium species (3.09%), Candida species (4.12%). The distribution of fungal species were categorized in Table 7.

10% KOH mount preparation used as a screening test for rapid diagnosis of fungal corneal ulcer showed 96.9% sensitivity and 96.8% specificity. From the Table 9 it was apparent that out of the 96 samples showing the presence of fungal elements in KOH mount 2 were negative for

culture. Of the 64 samples which did not show the presence of fungal elements in KOH mount 3 gave a positive culture report.

Antifungal susceptibility pattern of fungal isolates by Disk diffusion test showed that 55% Aspergillus fumigatus , 77% Aspergillus flavus , 76% Aspergillus niger, 62.5% Penicillium species, 60% Fusarium species were sensitive to Amphotericin B. 66% Aspergillus fumigatus, 88% Aspergillus flavus , 100% Aspergillus niger, 100% Penicillium species, 90% Fusarium were sensitive to Itraconazole. 70% Aspergillus fumigatus, 94% Aspergillus flavus, 100% Aspergillus niger, 100% Penicillium species, 80% Fusarium was sensitive to Voriconazole. 75% Candida species were sensitive to Fluconazole. (Table 10).

MIC of Amphotericin B by Agar dilution method, 39/58 (67.2%) Aspergillus species showed MIC of less than 2µg/ml. Among Aspergillus species, Aspergillus flavus and Aspergillus niger showed high sensitivity range compared with Asp.fumigatus. Penicillium species which showed MIC of less than 2 µg in 62.5 % (10/16) of isolates. Curvularia species showed 66 % (4/6) sensitivity and Acremonium species showed 100% sensitive range for Amphotericin B. (Table 11).

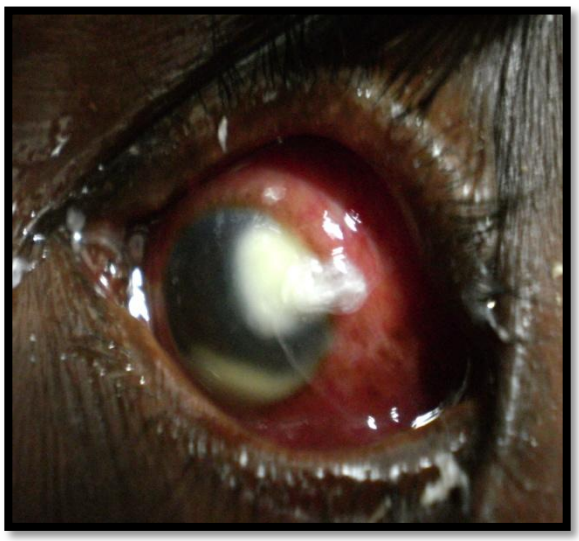
MIC of Itraconazole by Agar dilution method, isolates showed high sensitive range compared with Amphotericin B. 82% (47/58) of Aspergillus species, 90% (9/10) of Fusarium species showed less than 2µg/ml MIC value

for Itraconazole. Penicillium, Curvularia, Acremonium species showed 100% sensitivity to Itraconazole. (Table 12).

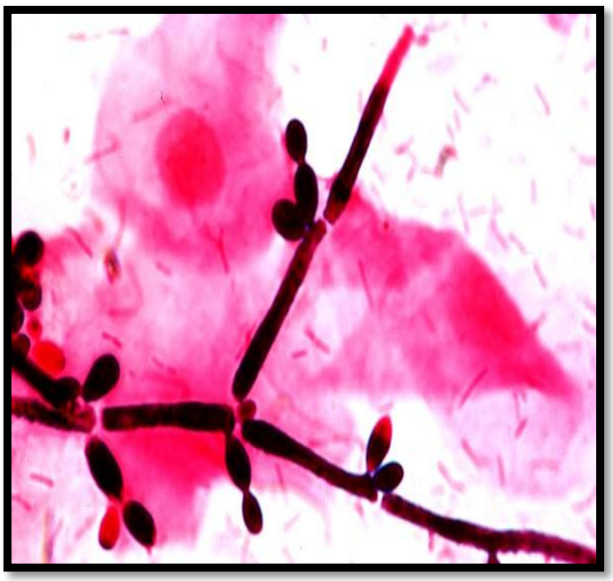
MIC determination by Broth microdilution method also showed that the MIC range was comparable with Agar dilution method. 70% of Aspergillus species, 75% of Penicillium species and 66% of Fusarium species showed sensitive range of MIC to Amphotericin B as showed Table 13. For Itraconazole 79% (46/58) of Aspergillus species showed MIC less than 2µg/ml. 100% sensitive range was noted in Penicillium, Curvularia, Acremonium species.(Table 14). A good correlation were observed between Agar dilution method and Broth microdilution method. (Table 15).

Azole group of drugs (Itraconazole, Voriconazole) showed high MIC_{50} range compared to Amphotericin B. (Table 16 & 17).

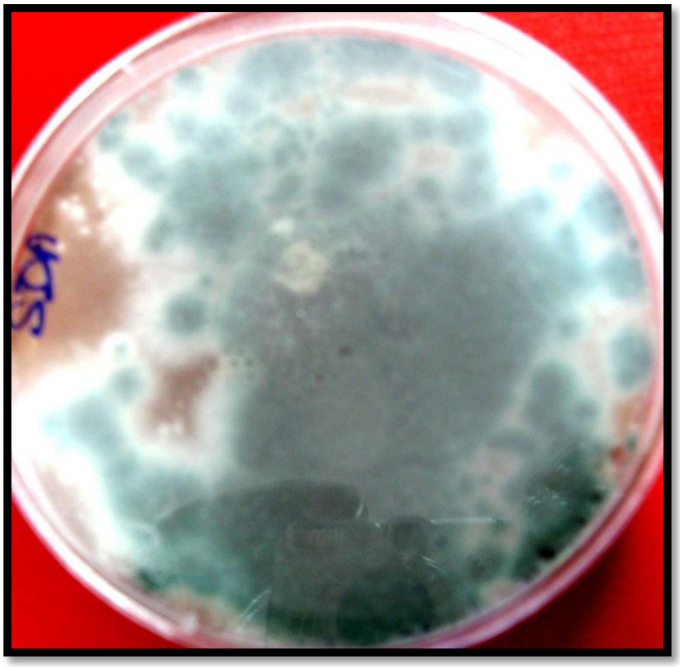
A CASE OF CORNEAL ULCER AND SPECIMEN TAKEN BY SCRAPING

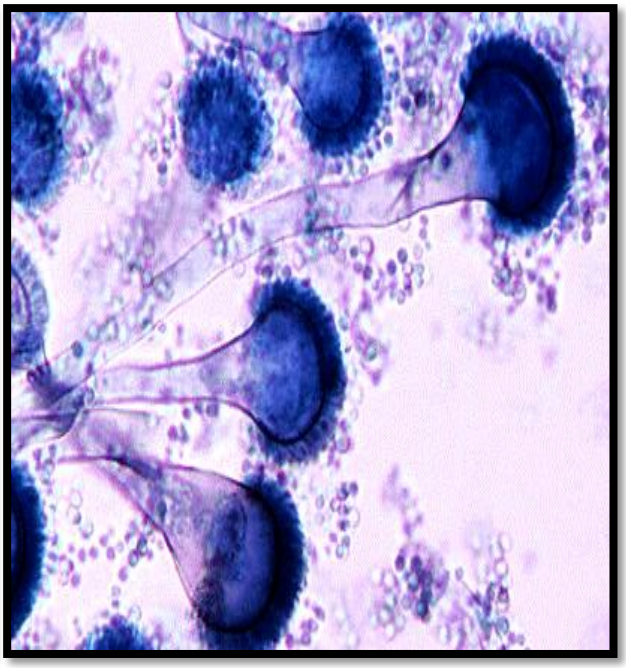


10% KOH MOUNT (FUNGAL ELEMENT) AND GRAM STAIN (YEAST CELLS)

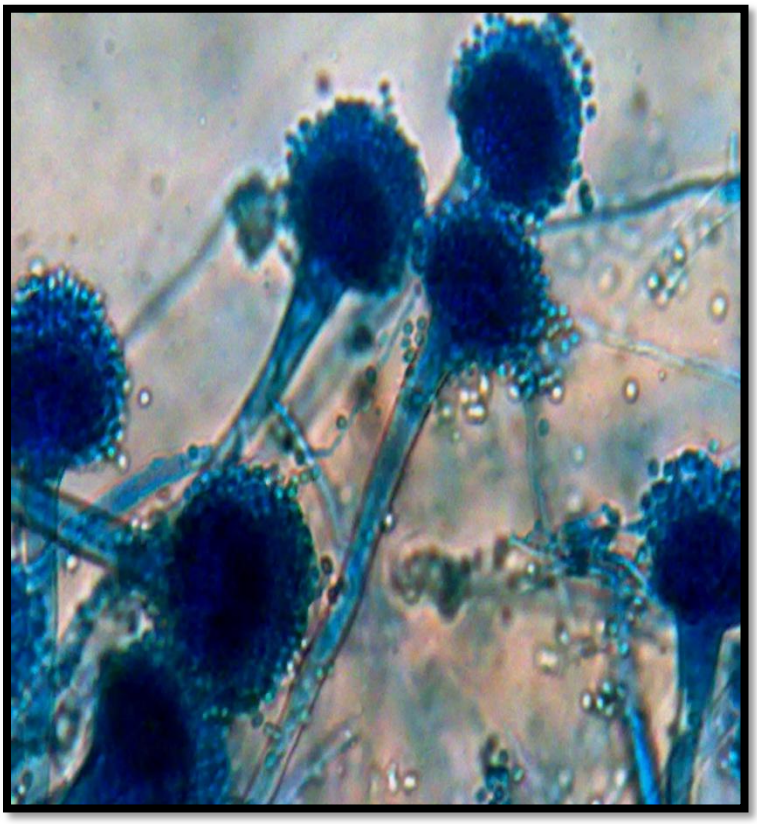


ASPERGILLUS FUMIGATUS ON SDA – LPCB MOUNT



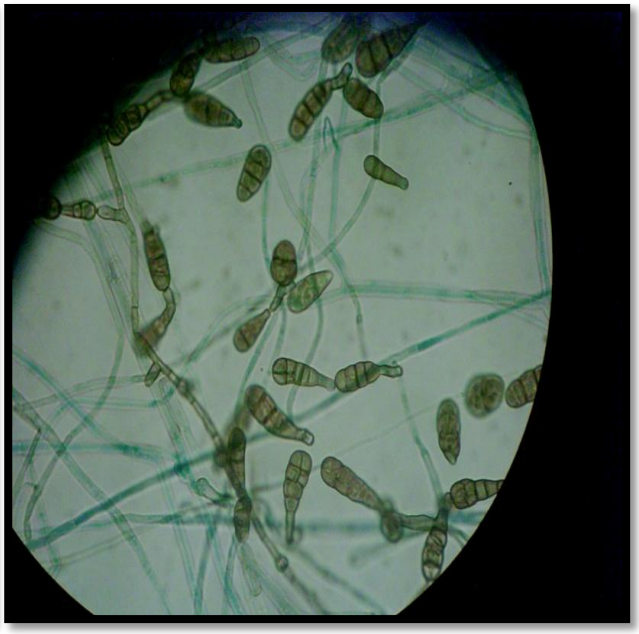


ASPERGILLUS NIGER ON SDA – LPCB MOUNT



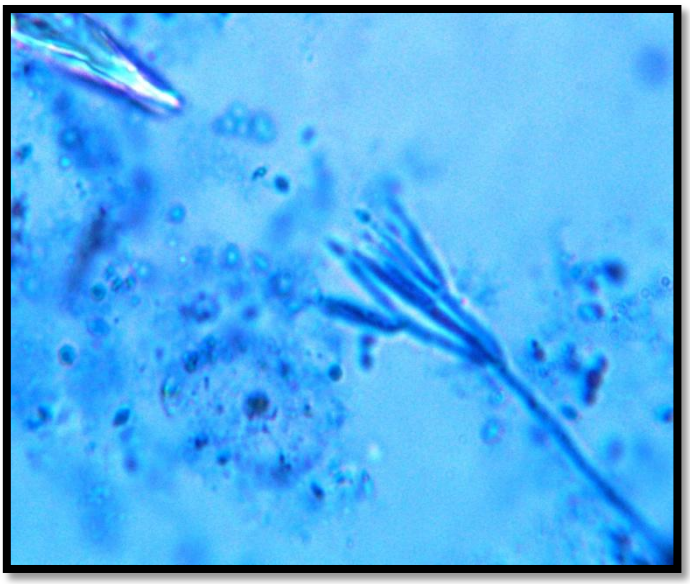
FUSARIUM ON SDA – LPCB MOUNT

CURVULARIA ON SDA – LPCB MOUNT



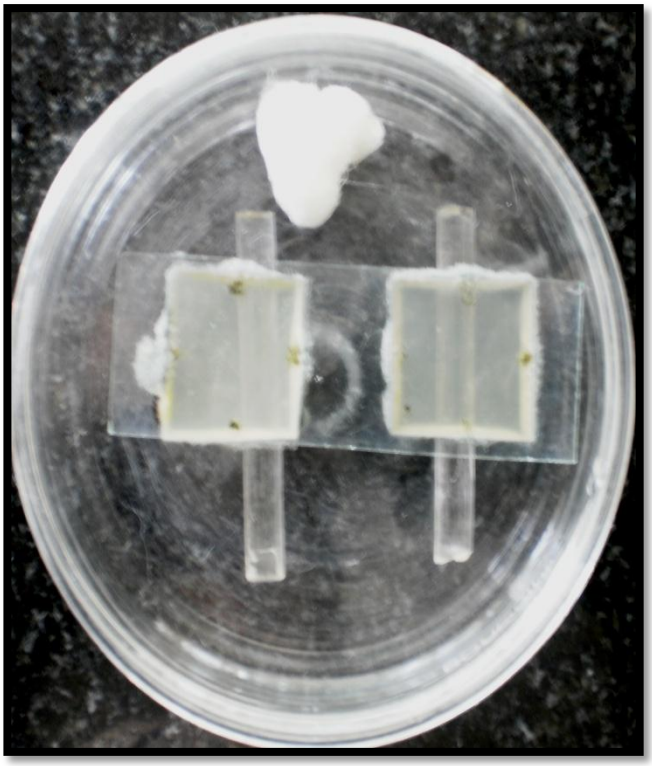
PENICILLIUM ON SDA – LPCB MOUNT

SLIDE CULTURE



ANTIFUNGAL SUSCEPTIBILITY TESTING

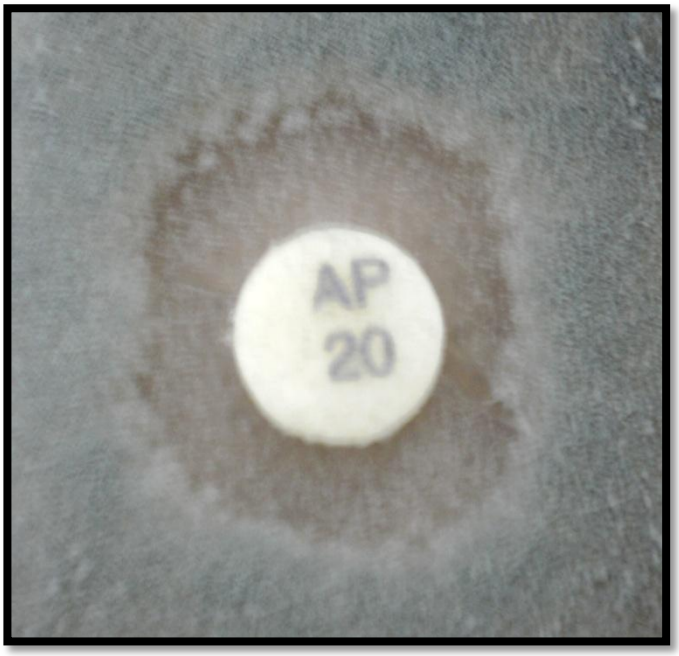
DISK DIFFUSION TEST



Ap : Amphotericin B It : Itraconazole Fu : Fluconazole

DISK DIFFUSION TEST

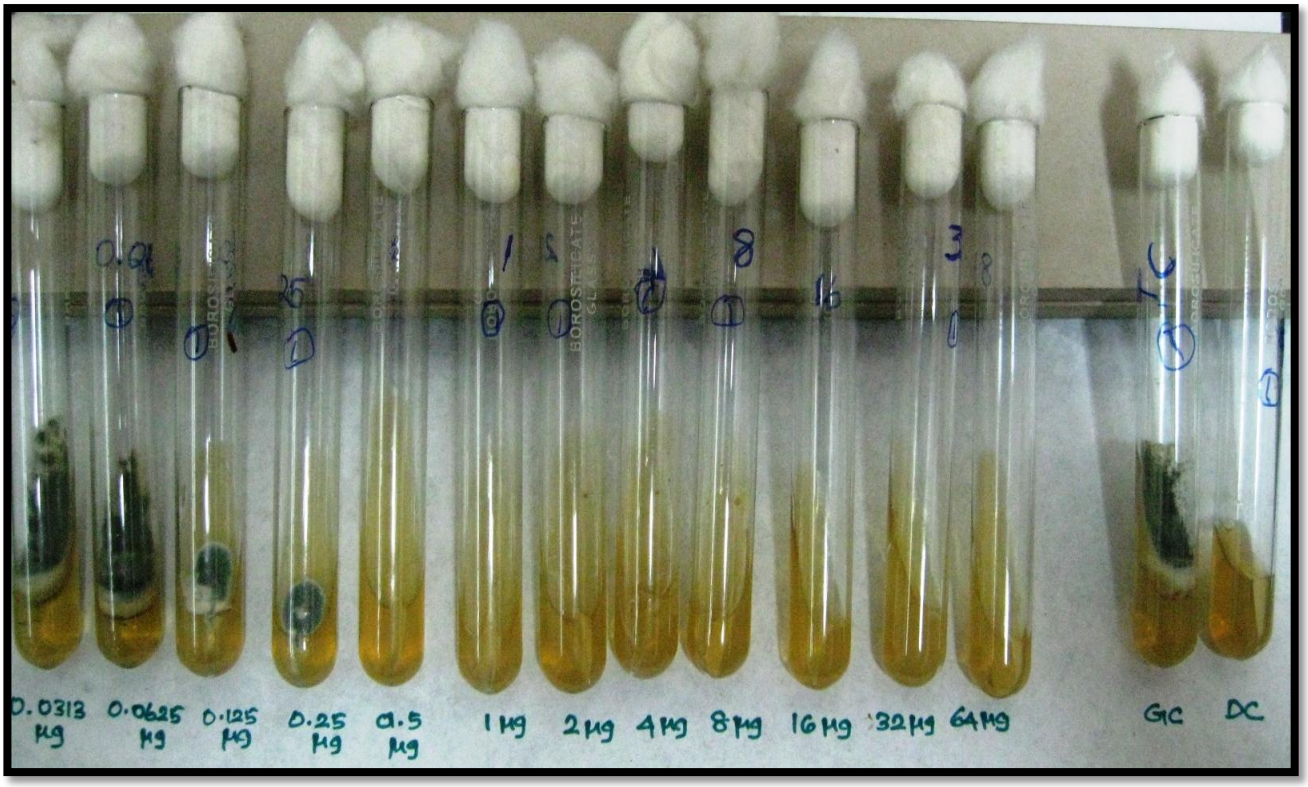
AMPHOTERICIN RESISTANCE BY PENICILLIUM RESISTANCE (ZONE SIZE <15MM)



DISK DIFFUSION TEST

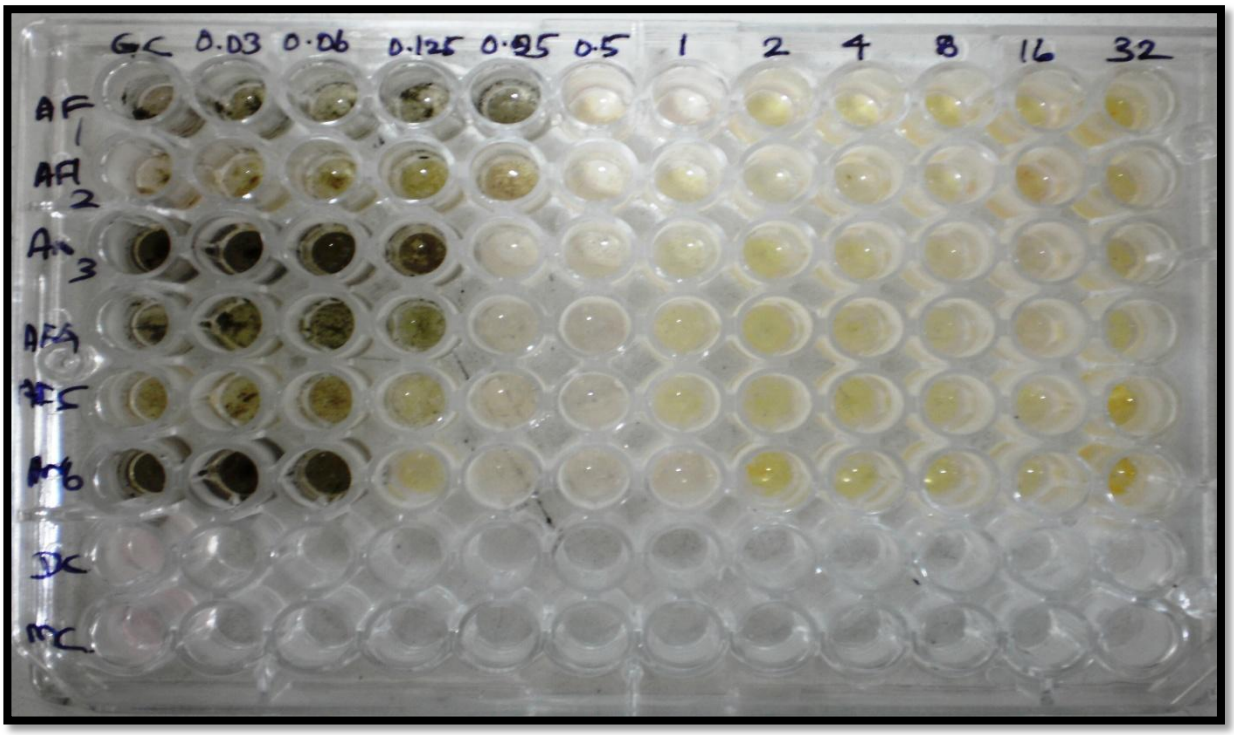
FLUCONAZOLE ASPERGILLUS FUMIGATUS

AGAR DILUTION METHOD (MIC) FOR PENICILLIUM SPECIES



AGAR DILUTION METHOD (MIC) FOR FUSARIUM SPECIES

BROTH MICRODILUTION METHOD (MIC)



MIC determination of Aspergillus species

Discussion

DISCUSSION

Infectious fungal corneal ulceration is a sight threatening condition with significant ocular morbidity due to their varied etiology. The etiological agents have to be correctly and promptly identified and treatment must be instituted at the earliest, if not it may result in permanent damage to the cornea with the permanent loss of vision.

The specimens for processing which were collected from the ulcers by scraping gave better results.

There have been numerous studies both in India and abroad on infectious corneal ulceration in the past 20 years. In all these studies it has been observed that there is changing spectrum of agents involved and predisposing factors in different geographical regions . Bharathi M J and Ramakrishnan R in 2002 studied the influence of risk factors, climate, and geographical variation of corneal ulcer in south India (Tirunelveli) and concluded that The risk of agricultural predominance and hot, windy climate makes fungal keratitis more frequent in south India.[7]

The present study showed the following results. Out of 160 corneal ulcers studied in detail, 97cases were showed culture positivity which accounts for 60.6%.This was similar to the study of Geetha K V et al in 2002,[104] which revealed 78% culture positivity .Bharathi M J et al in 2002[9]

and Khanal B et al in 2005[105] have reported 70% and 67.8% culture positivity in their studies respectively .

Though individuals of any age group can develop the lesion, people of certain age group are affected more. In this study the fungal corneal ulcer was found to be less common at the extremes of life and the commonest age group affected was 5th decade.

This observation correlates well with the study of Bharathi M J et al , 2003,[7] who reported higher prevalence among patients aged more than 50 years . The study of Chandar J et al, 1994,[106] also showed a higher prevalence of infected corneal ulcer in 51- 60 years age group. Parmar P *et al* in 2006 documented the prevalence of extremes of age from his study, shows with high severity and low prevalence.[70]

There was a male preponderance constituting two third of the study population. Similar findings were observed in the study of Chowdhary et al, 2005,[12] which revealed higher prevalence (68%),among males . Basak Samar K et al 2005,[10] and Lixen xie et al 2006[111] also reported male preponderance in their studies . In contrast Kottigadde Subbannayya et al , 1992[107] reported higher incidence (27%) in females than males (19%). L.C.Dutta et al,[16] V.C .Poria et al,[110] Sood et al[82] studies correlated with current study . Few other studies showed an equal incidence among both sexes (Mohan et al,[58] Upadhyal et al,[98] Halder K K et al[33]).

The wide variation seen among the reports on the spectrum of fungi causing corneal ulceration could be due to factors such as environment, habits and occupation of individuals, the season during which the studies were conducted, the nature of the predisposing factors, the use of antimicrobials/steroid and native medications to treat the ocular infections and the duration of the lesion.

The rural and urban distribution of corneal ulcer patients in this study revealed highest prevalence of infected corneal ulcers (65.4%) in people living in rural areas. This was similar to the study of Basak samar K et al , 2005,[10] in which 78.5% of the patients were from rural areas .The study of Bharathi M J et al 2003,[7] and Chander J et al,1994,[106] also showed higher prevalence of infected corneal ulcers in patients from rural background .

Corneal ulcers have been known to occur following corneal trauma which may be agricultural or accidental injury, thermal injury, iron particles, stone etc.,

In this study, ocular trauma was the most important predisposing factor. A definite history of antecedent corneal injury was recorded in 41.23% of the patients, which was in agreement with the studies of Gopinathan et al 2002,[30] and Barak Samar K et al 2005.[10] In their studies history of ocular trauma was noted in 54.5% and 83% of patients respectively . The studies conducted in abroad by Norina T J et al 2008,[108] and Laspinal

F et al , 2004,[109] also revealed history of ocular trauma in 62% and 50% of their patients .

In studying the different agents of trauma such as paddy dust, wood dust etc, 15 cases (37.5%) were associated with history of injury by vegetable matter. This correlates with the study of Bhasak Samar K et al, 2005,[10] according to which 59.6% patients had corneal injury with vegetative matter .

The above observations clearly show that in developing countries , where agricultural work is more common ,vegetative matter induced ocular trauma is the major cause of infectious corneal ulceration .

Considering the seasonal variation of Mycotic keratitis, is more common during the hot, dry, windy season. The incidence of fungal corneal ulceration is more during this period. 47.5% of the samples taken during that period in this study shown in master chart. The seasonal incidence of Mycotic keratitis correlate with the report by Liesegang *et al,*[46] as the incidence of Mycotic keratitis was higher during the the hot, dry, windy season (51.67%) compared to that during rainy season (31.67%).

Fungal corneal ulcer also results as a sequelae to certain operation like cataract surgery, penetrating keratoplasty or even after wearing contact lenses. Ainbinder ,DJ, Parmley VC ,et al 1998[2] documented the fungal corneal ulcer after penetrating keratoplasty. Certain disease where patients are immunologically compromised like diabetes mellitus, Hansen's disease, Bell's palsy etc also predispose to corneal ulceration. Local application of

steroids, antimicrobials or native medicine may also predispose to infection of cornea leading to ulceration. In this study 10.5% of cases gave history of prior antifungal / steroid use and 7.2% with Hansen's disease, 5.2% had postoperative ulcer which correlate with study by Sood *et al.*[82]

According to this study aetiological agents were isolated in 97 (60.6%) samples. These observation were similar to the study of Basak Samar *et al* in 2005[10] which revealed 62.7% of fungal growth and study by Khanal B *et al* in 2005[105] conducted in Nepal showed 42.7% growth positive for fungi.

In contrast the study conducted by Norina T J *et al* in 2008[108] in Malaysia revealed only 13.8% and Laspia F *et al* in 2004[109] in Peraguay, have reported 26% of fungal growth isolated from corneal ulcer patients. These study observations clearly show that fungal corneal ulcer is more common in developing countries and have wide range of geographical variation.

Among the fungal isolates in this study 58 (59.7%) were Aspergillus species followed by Penicillium species 16 (16.49%) and Fusarium species 10 (10.3%) and the remaining 13 (13.4%) isolates were Curvularia species, Acremonium spp. Candida spp. It is evident from our study that Aspergillus species was by far the commonest filamentous fungi causing corneal ulcer.

The dominant role of Aspergillus species in corneal ulcer has been reported in the studies of Basak Samar K *et al* in 2005 and Khanal B *et al* in 2005.[105] In their studies the commonest pathogen was Aspergillus species

followed by Fusarium species. Zimmerman E.L *et al* reported Aspergillus was the commonest isolates in corneal ulcer.[103]

In the study of Lixen *et al* in 2006[111] and Prashant *et al* in 2007,[112] Fusarium species was found to be the most common fungi isolated. In this study Fusarium was isolated only in 10.3% of samples next to Aspergillus spp. This may be explained by differences in climate and natural environment.

In the present study, Acremonium species was isolated in 3.09% of samples, which was closer to the study of Chander K *et al* in 1994,[106] where Acremonium species accounted for 6.6% of the isolates.

In evaluating the screening tests for rapid diagnosis of aetiological agents in infectious corneal ulcers, 10% potassium hydroxide (KOH) mount Gram stain examination of the corneal scrapings were analyzed.

10% KOH mount examination showed a sensitivity of 96.9% and a specificity of 96.8%. This correlates with the study of Vajpayee R B et al 1993,[99] which revealed 94.3% sensitivity of 10% KOH mount examination. Bharathi M J et al, 2007,[9] reported 99% sensitivity and 1.5% false positive rate of KOH wet mount preparation .The false positive rate of KOH smear in the present study was 1.4% . Although culturing of microbial pathogens is considered to be the gold standard, direct microscopic evaluation of smear provides immediate information about the aetiological agents and aid in early initiation of microbial therapy.

In the present study, antifungal susceptibility was performed for Amphotericin B, Itraconazole and Fluconazole by disk diffusion method, agar dilution method, broth microdilution method (CLSI guidelines).

Antifungal susceptibility pattern of fungal isolates by Disk diffusion test showed that 55% Aspergillus fumigatus , 77% Aspergillus flavus , 76% Aspergillus niger, 62.5% Penicillium species, 60% Fusarium species were sensitive to Amphotericin B. 66% Asp.fumigatus, 88% Asp.flavus , 100% Asp.niger, 100% Penicillium species, 90% Fusarium were sensitive to Itraconazole. 70% Aspergillus fumigatus, 94% Aspergillus flavus , 100% Aspergillus niger, 100% Penicillium species, 80% Fusarium were sensitive to Voriconazole. 75% Candida species were sensitive to Fluconazole.

MIC of Amphotericin B by Agar dilution method, 39/58 (67.2%) Aspergillus species showed MIC of less than 2µg/ml. Among Aspergillus species, Aspergillus flavus and Aspergillus niger showed high sensitivity range compared with Aspergillus fumigatus. Penicillium species show MIC of less than 2 µg in 62.5 % (10/16) of isolates. Curvularia species shows 66% (4/6) sensitivity and Acremonium shows 100% sensitive range for Amphotericin B. In a study by Therese K. *et al* showed 86% Aspergillus species sensitive to Amphotericin B and 6.4% resistant to it.[96]

MIC of Itraconazole by Agar dilution method, isolates showed high sensitivity range compared with Amphotericin B. 82% (47/58) of Aspergillus species, 90% (9/10) of Fusarium species show less than 2µg/ml MIC value

for Itraconazole. Penicillium, Curvularia, Acremonium species show 100% sensitivity to Itraconazole. Ray A in 2002[81] studied the efficacy of Itraconazole showed 80% success rate of Itraconazole therapy in Aspergillus species.

MIC determination by Broth microdilution method also showed the MIC range comparable with Agar dilution method. 70% of Aspergillus species, 75% of Penicillium species, 66% of Fusarium species showed sensitivity range of MIC to Amphotericin B. For Itraconazole 79% (46/58) of Aspergillus species show MIC less than 2µg/ml. 100% sensitivity range was noted in Penicillium, Curvularia, Acremonium species.

A good correlation observed between Agar dilution method and Broth microdilution method. Results obtained by Asit R. Banerjee *et al* in 2001[5] with sensitivity for Itraconazole to Aspergillus, Penicillium and Fusarium species showed 77% sensitivity and 23% did not respond well to treatment among that 27% resistance shown by Fusarium species.

Summary

SUMMARY

- Totally 160 infectious corneal ulcers were studied in detail. Aetiological agents were isolated in 97 (60.6%) cases. Majority of the isolates were fungal agents belonging to the genus Aspergillus (59.79%) followed by Penicillium species (16.49%) and Fusarium species. (10.3%).
- Male preponderance was observed (62.8%) in this study.
- The age group most commonly affected was between 51& 60 years comprises 41.2% of total cases.
- Incidence of infectious corneal ulcer was more in rural population than urban population.
- Trauma with vegetative matter was found to be the most common predisposing factor (40%) in the development of infectious fungal corneal ulcers. So spectrum of microbial keratitis varies with geographical location influenced by the local climate and occupational risk factors.
- 10% KOH mount found to be highly sensitive as rapid screening tests for diagnosing fungal corneal ulcers with sensitivity of 96.9%. The sensitivity of 10% KOH mount correlate with culture reports.

- Aspergillus species were most commonly isolated from corneal ulcer patients.(59.7%). In that Aspergillus fumigatus accounts for 46.5%, Aspergillus flavus 31% and Aspergillus niger 22.4%.
- 88.6% of fungal isolates were sensitive to Voriconazole. 87.6% of fungal isolates were sensitive to Itraconazole. 67% of isolates were sensitive to Amphotericin B. 95% fungal isolates were resistant to Fluconazole by Disk diffusion method.
- Totally 63.9% of isolates exhibited sensitivity range for Amphotericin B and 83.5% of isolates exhibited sensitivity range for Itraconazole in Agar dilution method.
- In Broth microdilution method 67% of isolates exhibited sensitivity range for Amphotericin B and 82.47% of isolates exhibited sensitivity range for Itraconazole.
- "**Prevention is better than cure**" strategy to be followed promptly to reduce accidental injury and to create awareness among public in regards with early presentation to the hospital to avoid inadvertent complications.
- **"Goal to vision 2015"** programme put forth by the Institute of Ophthalmology, aims to reduce the incidence of corneal ulcer and its complications, thereby making the world a colourful place to live.

Conclusion

CONCLUSION

The following are the conclusions derived from the present study on the aetiopathogenesis of corneal ulcers.

- Corneal ulcers are more common during 5^{th} decade of life with male preponderance with rural background.
- A variety of fungal isolates can cause infectious corneal ulceration in which Aspergillus fumigatus was the most common fungal species isolated which was susceptible to Amphotericin B, Itraconazole, Voriconazole.
- Among the various predisposing factors trauma in farm workers plays a major role in producing corneal ulceration with seasonal variation.
- Microscopy and culture (gold standard) should be the dictum for every case of corneal ulcer investigation in the laboratory.
- Precise identification of the causative organisms and timely institution of appropriate antifungal therapy based on the prevailing sensitivity pattern of the fungal isolates could save the eye from this preventable cause of blindness which was carried out by Media education and audio visual aids to create public awareness regarding “vision and vulnerability to infection”.

Annexures

ETHICAL COMMITTEE

GOVT. KILPAUK MEDICAL COLLEGE, KILPAUK, CHENNAI- 10.

Venue: PANAGAL HALL, KMC
Dt: 26.11.2009

CHAIRPERSON
Prof. Dr.V.KANAGASABAI, MD.,
The Dean
Govt. Kilpauk Medical College, Chennai-10

Sub: Ethical Committee project work - approved.
Ref: Lr.No.3944/P&D/09 Dt. 17.11.09.

With above reference, the Institutional Ethical committee meeting for the following students was conducted at our Institution on 26.11.09.

1.	Dr.Uma S. Pandian IIIr Yr (Micro)	Microbiological profile of Diabetic foot ulcers.
2.	Dr.V.Vasanthapriyan.M.R	Fungal isolates in human corneal ulcer
3.	Dr.B.Ravichandran	Study of Bacteriological profile in postoperative would infections.

We are glad to inform you that at the Ethical Committee meeting the documents were discussed and the above short term projects are Ethically approved.

21/12/09

CHAIRPERSON
Prof. Dr.V.KANAGASABAI, MD.,
The Dean
Govt. Kilpauk Medical College,
Chennai-10.

To
The Individuals

PROFORMA

Name: Age: Sex: M/F

OP/IP no Date of sample collection:

Occupation:

Place of work : Rural / Urban Duration of work:

Address : Socioeconomic status:

CORNEAL ULCER DETAILS

Affected Eye: R / L Duration:

Vision : RE: LE:

Family history:

H/O Trauma: Y/N Details: Nature of trauma/ place of trauma/ material

Co-morbid conditions:

(a)Hypertension : Y/N Duration: Treatment:

(b)Diabetes Mellitus : Y/N Duration: Treatment:

(c)Previous ulcer : Y/N if yes (i)Affected Eye: R/L

(ii)Duration:

(iii)Treatment details:

Ophthalmic surgery : if any specify Duration:

Hospital stay:

Smoking : Y/N Duration:

Alcohol : Y/N Duration:

H/O Hospitalisation: Y/N if yes Duration:

Recent Antifungal usage: Topical/oral/injectable Duration:

ULCER CHARACTERISTICS

- Affected Eye: RE /LE
- Ulcer size:
- Single / Multiple
- Position:
- Any discharge:

MICROBIOLOGICAL PROFILE

- Gram stain:
- KOH mount:
- Fungal culture: colony morphology: Obverse:

  Reverse:
- LPCB mount:
- Antifungal susceptibility report: Sensitive / Resistant

  MIC range / MIC_{50} / MIC_{90}

OUTCOME OF PATIENT

- Time taken for healing:
- Recurrence:
- Reinfection:
- Change of Antifungals:
- Readmission:

APPENDIX

A. STAIN & REAGENTS :

1. 10% KOH :

Potassium hydroxide	:	10 g
Glycerol	:	10 ml
Distilled water	:	80 ml

2. GRAM STAIN :

Methyl violet (2%)	:	10g methyl violet in 100 ml absolute alcohol 1 lit of distilled water (Primary stain)
Grams Iodine	:	10 g Iodine in 20 g KI (fixative)
Acetone	:	Decolorising agent
Carbol fuchsin 1%	:	counter stain

3. LACTOPHENOL COTTON BLUE :

For the staining and microscopic identification of fungi

Cotton Blue (Aniline Blue)	:	0.05 g
Phenol Crystals (C6H5O4)	:	20 g
Glycerol	:	40 ml
Lactic acid (CH3CHOH COOH)	:	20 ml
Distilled water	:	20 ml

Method of preparation : This stain is prepared over two days.

1. On the first day, dissolve the Cotton Blue in the distilled water. Leave overnight to eliminate insoluble dye.

2. On the second day, wearing gloves add the phenol crystals to the lactic acid in a glass beaker. place on magnetic stirrer until the phenol is dissolved.

3. Add the glycerol.

4. Filter the Cotton Blue and distilled water solution into the phenol/glycerol/lactic acid solution. Mix and store at room temperature.

B.MEDIA USED :

1. **BRAIN HEART INFUSION BROTH :**

Sodium citrate	:	1g
Sodium chloride	:	4g
Sodium phosphate	:	5g
Dextrose	:	10g
Peptone	:	10g

Brain heart infusion :

Brain infusion broth	:	250 ml
Heart infusion broth	:	750 ml
Sodium polyenonthal sulphonate	:	0.25 g

Obtain ox brain and heart. Remove all fat from heart and cut into small pieces and grind. Add distilled water three times and keep it at 4˚c overnight.

From the brain remove the meninges fully and then weigh. Add distilled water and mash by using hand. Keep cooler overnight. Next morning boil the brain and heart separately for 30 minutes. Then filter through cotton layer. Measure each broth separately. Mix both infusions and the remaining ingrediants. Dissolve well and adjust the pH to 7.4-7.6.

Autoclave at 121˚c for 15 minutes. Filter through filter paper and distribute in screw capped bottles in 50 to 100 ml amounts. Autoclave again at 115˚c for 10 minutes.

2. SABOURAUD'S DEXTROSE AGAR :

Dextrose	:	20g
Neo Peptone	:	10g
Agar	:	20g
Distilled water	:	1000ml

pH : 6.8 ± 0.2

Suspend the ingredients in water, dissolve by heating to a boil and dispense in approximately 20 ml amounts in cotton plugged 25x150 mm test tubes with antimicrobial agent (Gentamicin 20 mg) added after heating the medium and before autoclaving at 121˚c for no longer than 15 minutes. Slant was allowed to harden and refrigerated.

Note : Cycloheximide was not added to the media since it is known to inhibit ocular fungal pathogen.

3. POTATO DEXTROSE AGAR :

Potato	:	200g
Dextrose	:	20g
Agar	:	20g
Water	:	1 litre

pH : 5.6 ± 0.2

Scrub, slice and boil potatoes in 100 ml of distilled water for one hour. Filter infusion through gauze and add agar and boil till it dissolve completely. Add dextrose and make upto one litre by adding distilled water. Sterilize by autoclaving at 15 pounds pressure at 115˚c for 30 minutes. Cool to 50˚c and approximately 20 ml into Petridishes.

4. MULLER HINTON AGAR :

Beef infusion	:	300 ml
Caesein hydroxylate	:	17.5g
Starch	:	1.5g
Agar	:	10g
Distilled water	:	1 litre

pH : 7.4

Emulsify the starch in a small amount of cold water, pour into the beef infusion and add casein hydroxlysate and add agar. Make up the volume to 1 litre with distilled water. Dissolve the constituents by heating gently at 100˚c with agitation. Filter if necessary. Adjust the pH to 7.4. dispense in screw-capped bottles and sterilize by autoclaving at121˚c for 20 minutes.

5. RPMI 1640 MEDIUM :

Commercially purchased RPMI 1640 media supplement with 0.3g of L-glutamate per litre without sodium bicarbonate (powder). Dissolve the powder in Nuclease free water . The medium was sterilized by filtering through a sterile membrane filter with a porosity of 0.22 microns. The pH was adjusted to 7.0. MOPS buffer was used.

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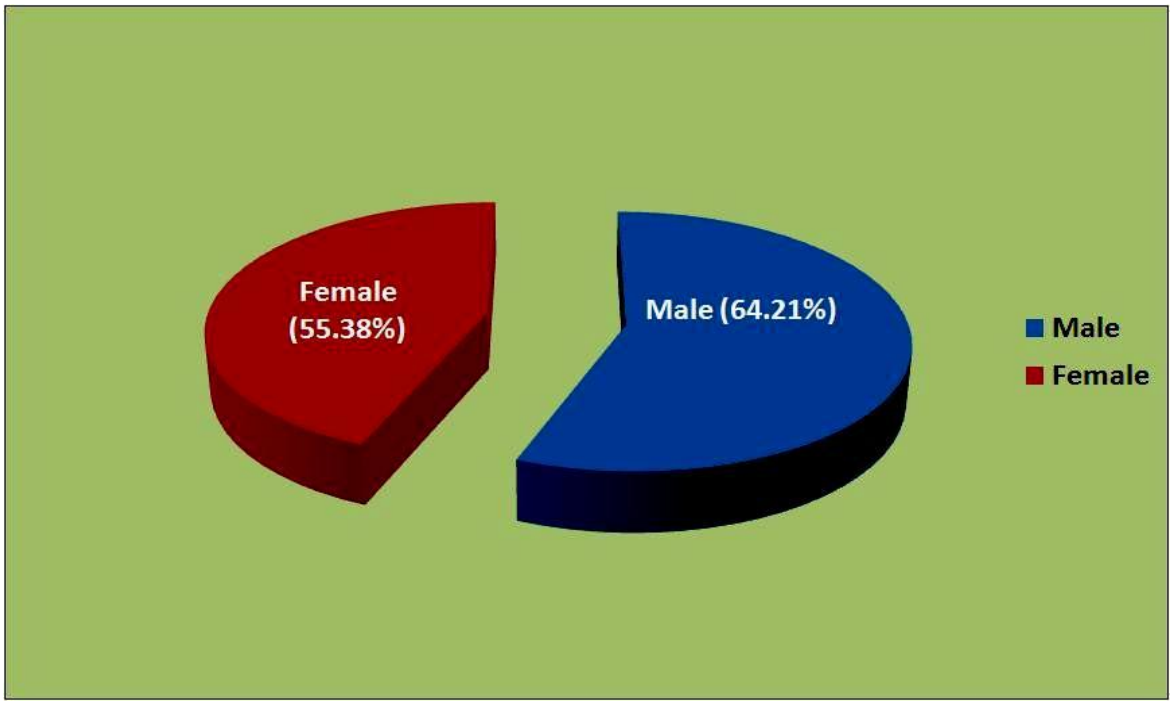
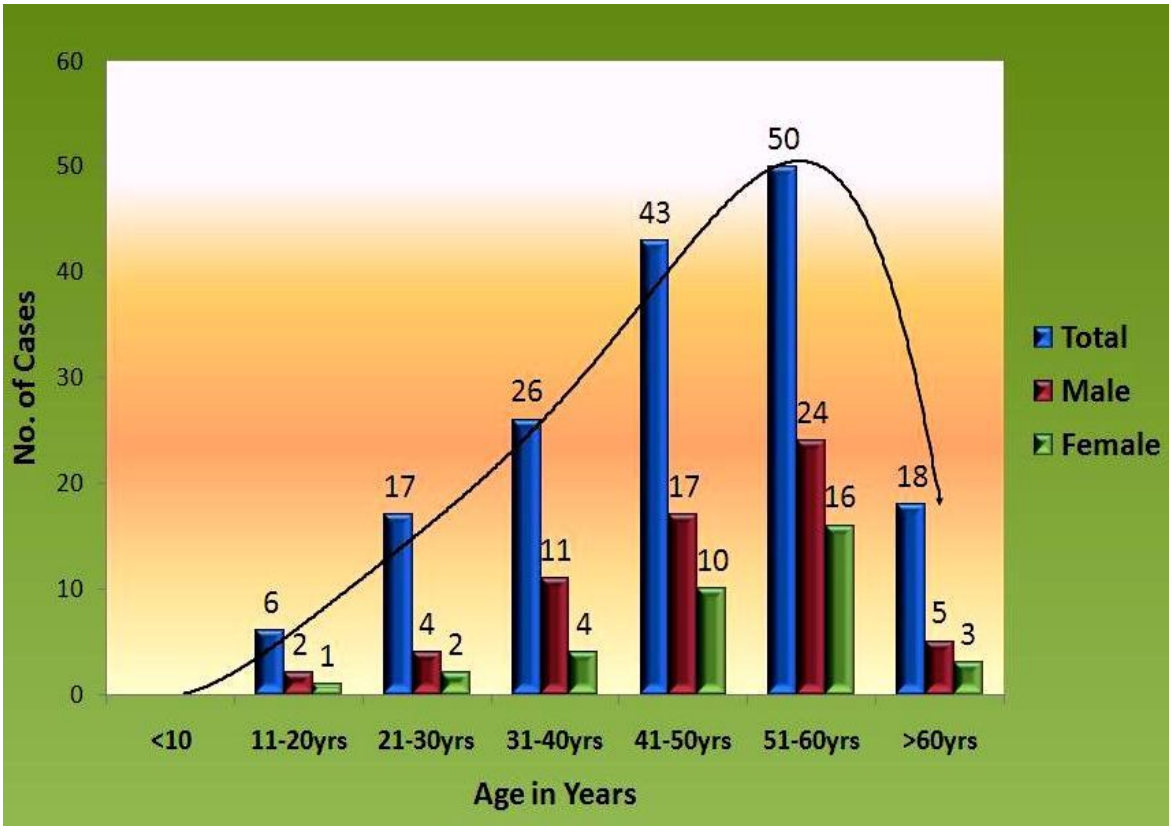
GENDER DISTRIBUTION OF INFECTIOUS CORNEAL ULCER

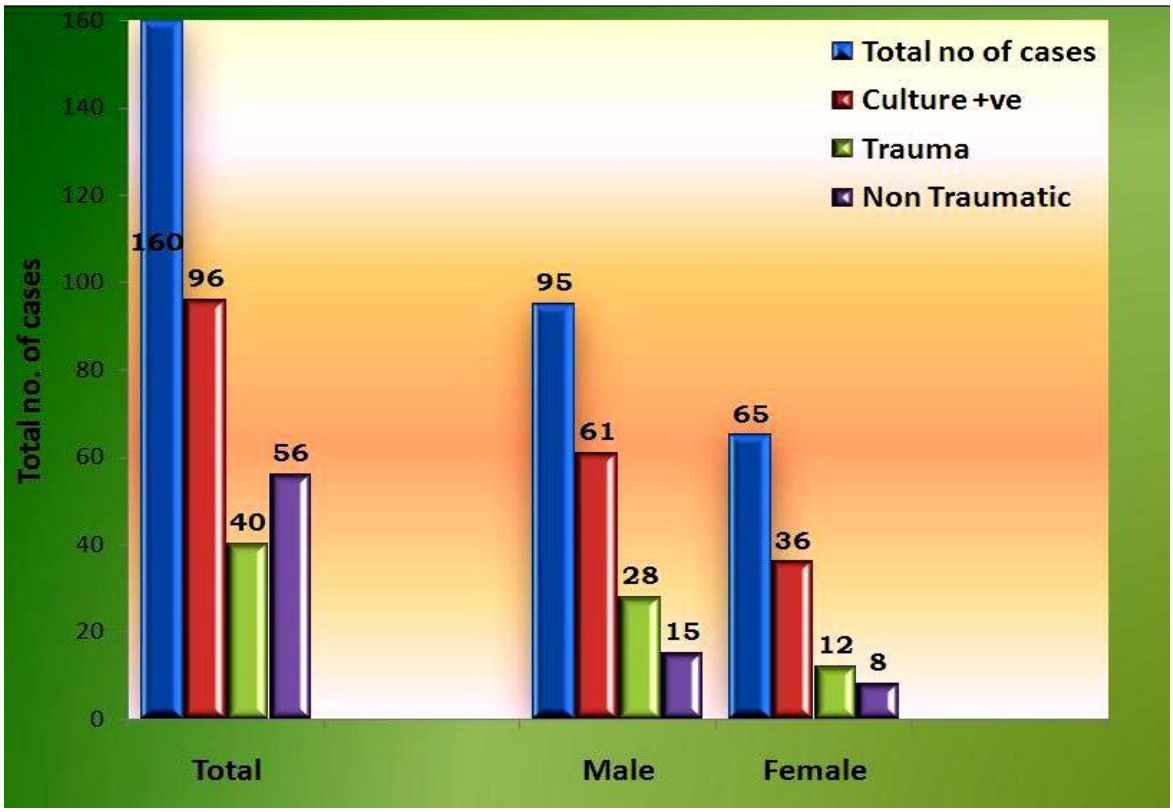


AGE DISTRIBUTION OF INFECTIOUS CORNEAL ULCER

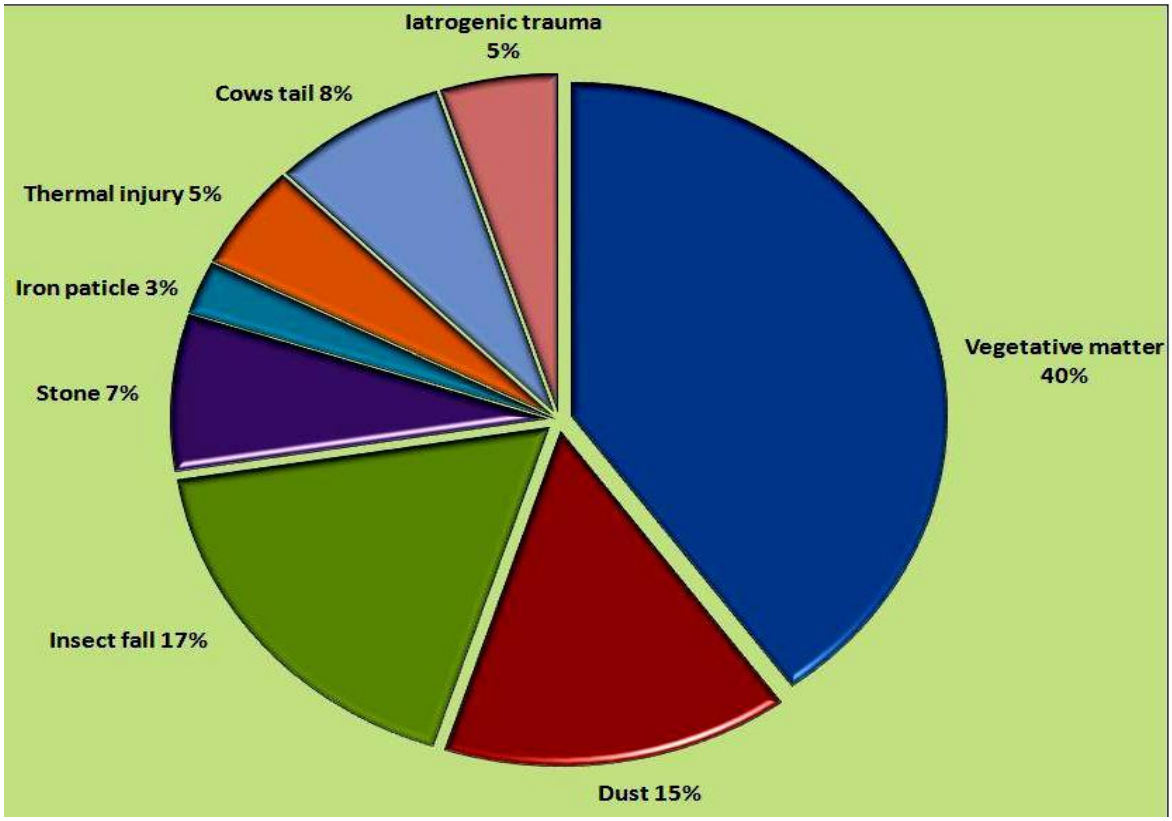


Prevalence of corneal ulceration was more common in 51-60 yrs of age group.

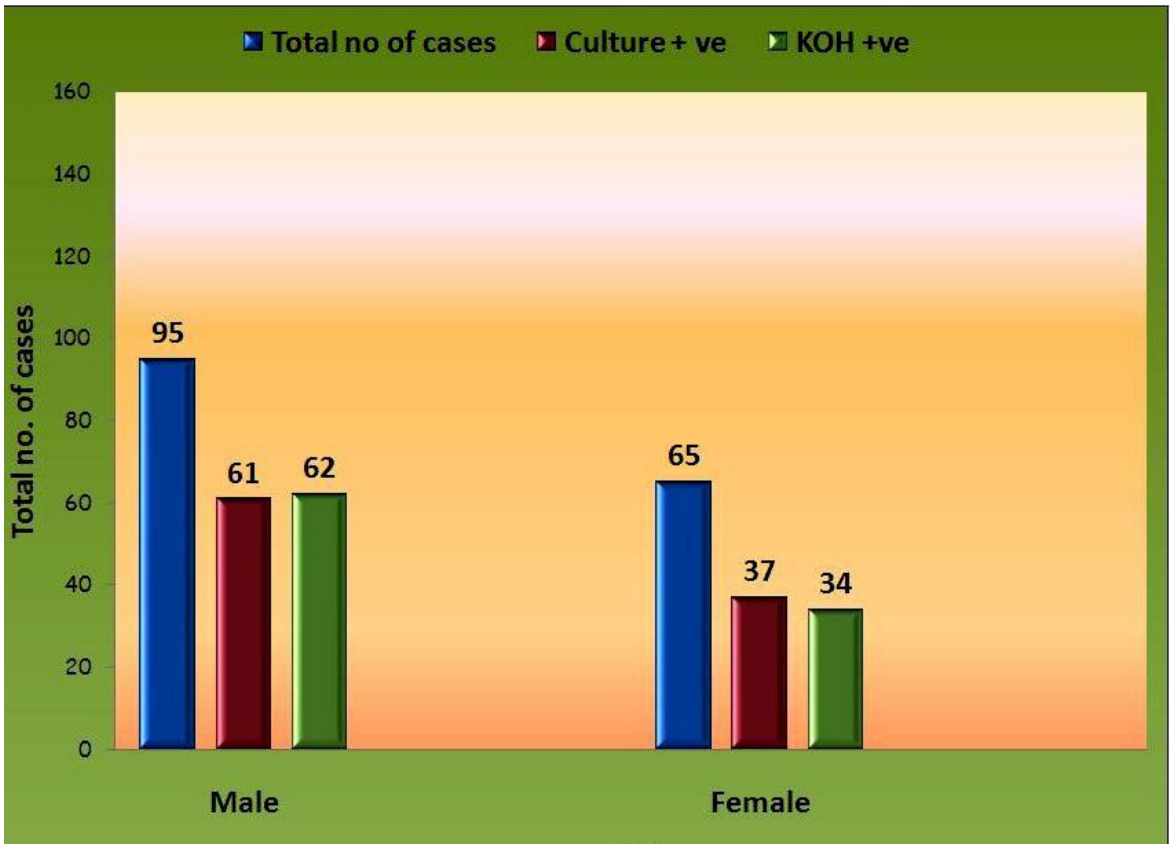
DISTRIBUTION OF PREDISPOSING FACTORS IN CORNEAL ULCER



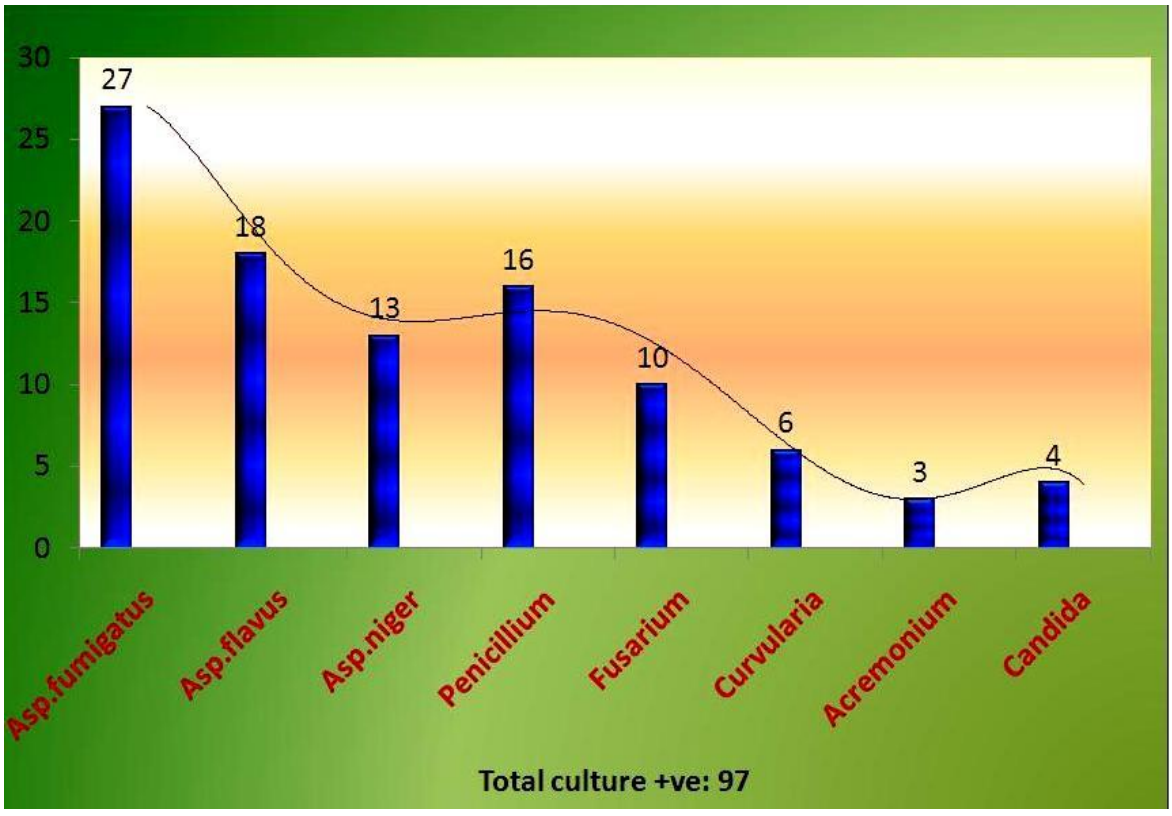
DISTRIBUTION OF TRAUMA FACTORS IN CORNEAL ULCER



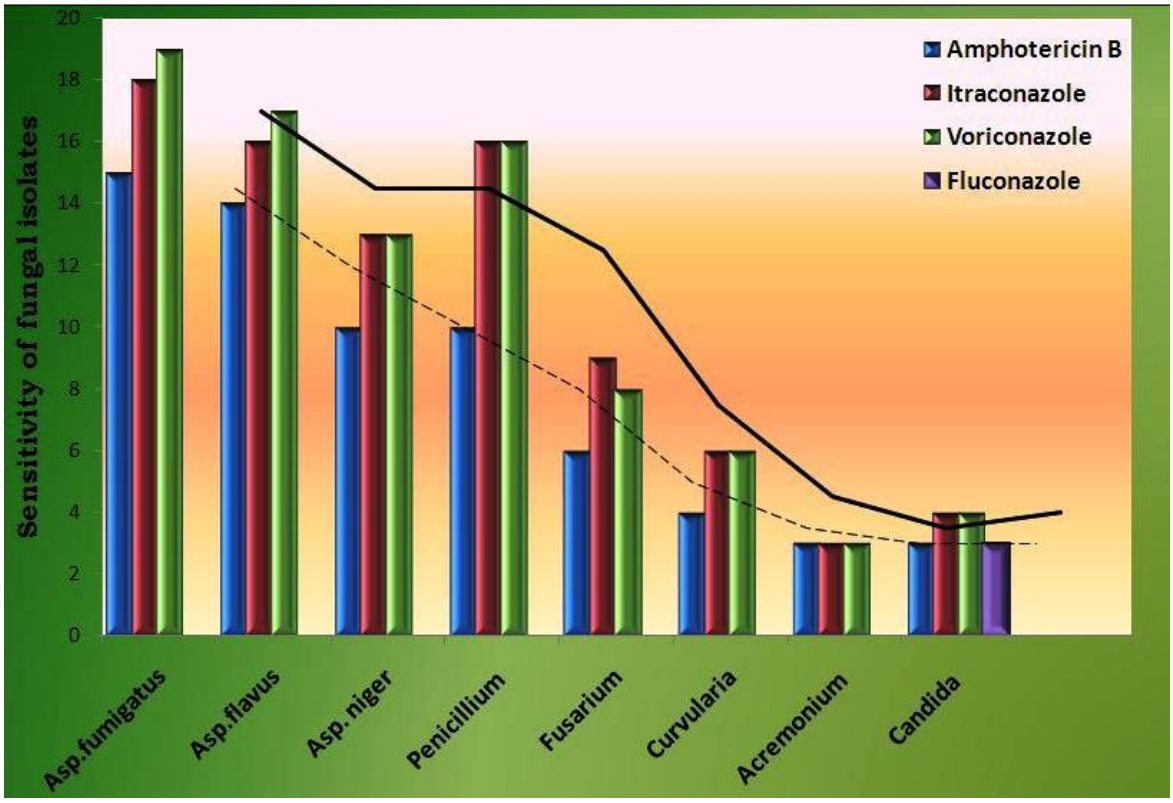
GENDER DISTRIBUTION WITH CULTURE POSITIVITY AND 10% KOH MOUNT



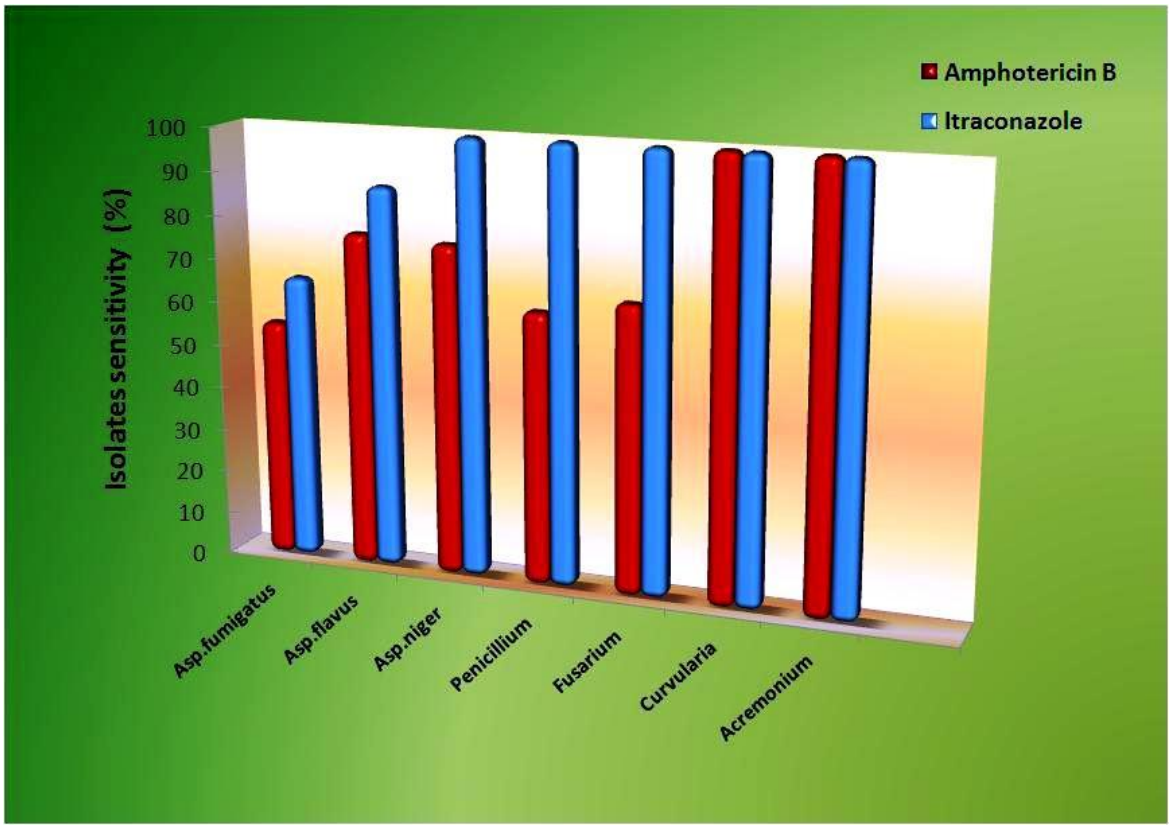
DISTRIBUTION OF FUNGAL AGENTS CAUSING CORNEAL ULCER



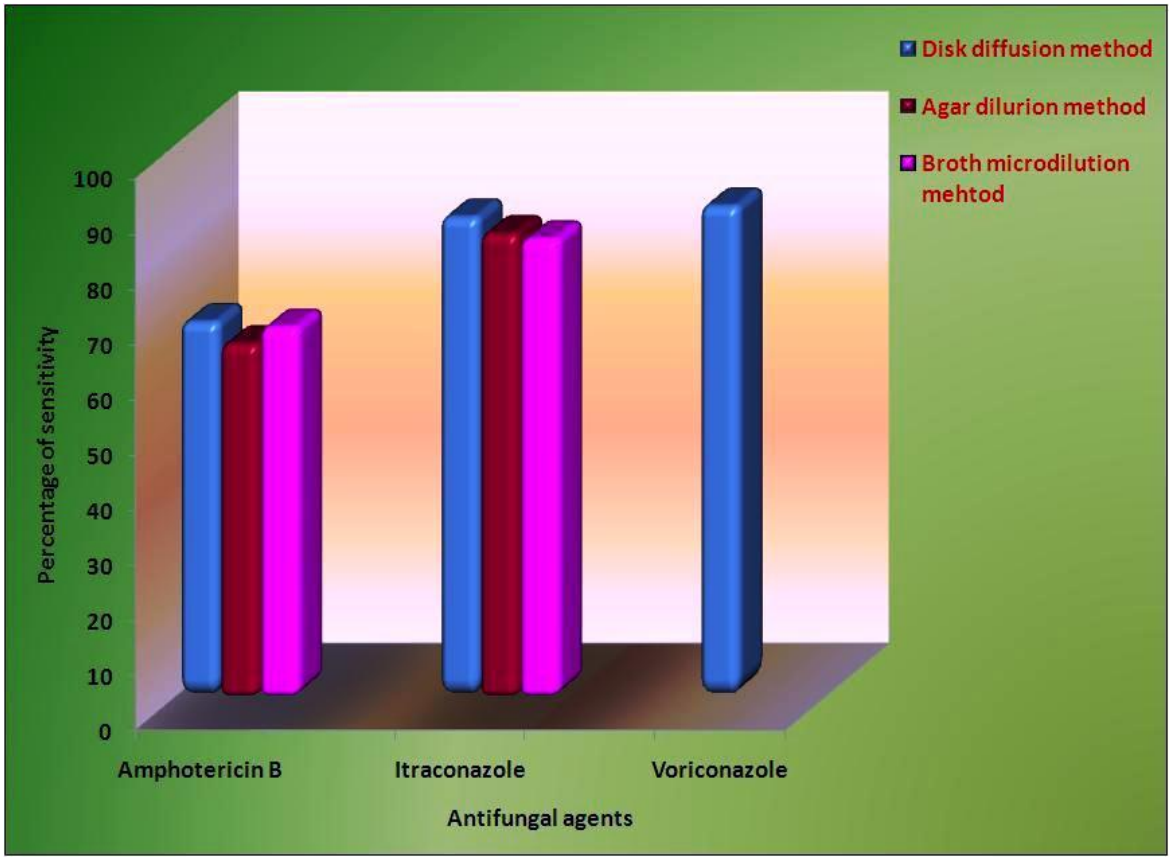
ANTIFUNGAL SUSCEPTI BILITY TESTING DISK DIFFUSION METHOD

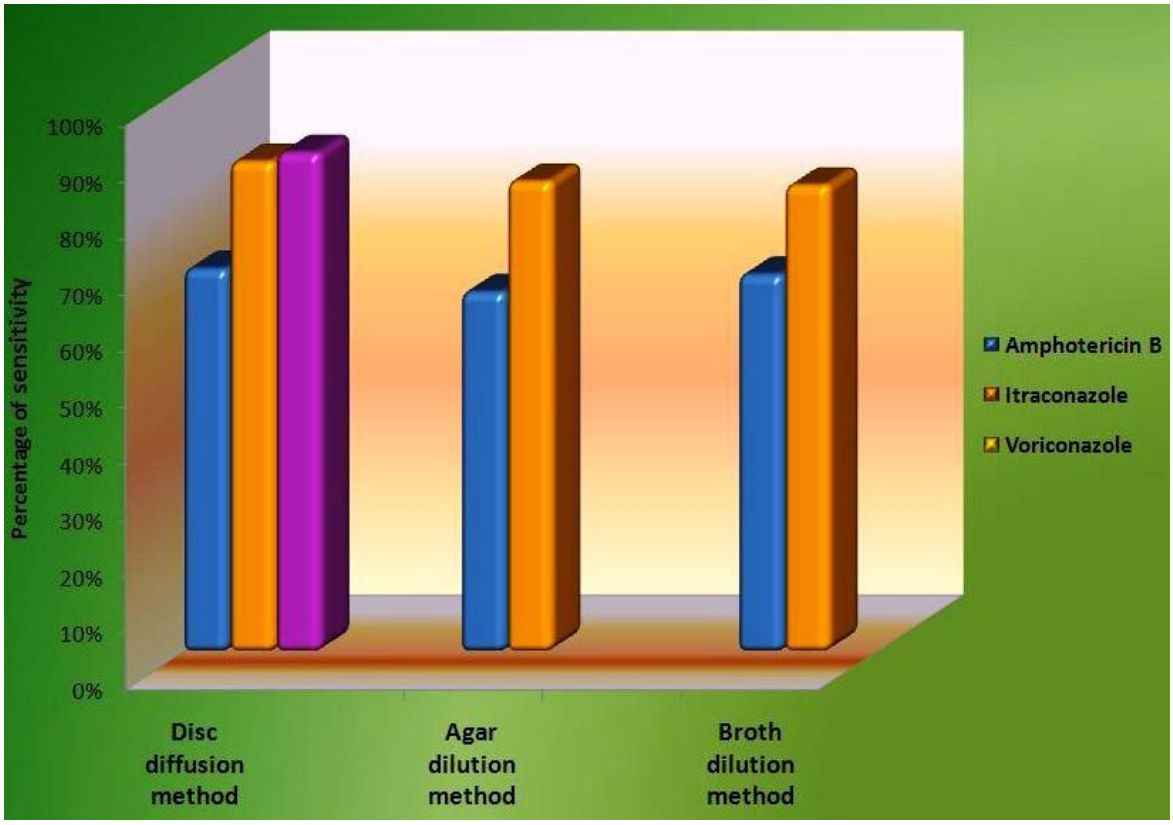


MINIMUM INHIBITORY CONCENTRATION AGAR DILUTION METHOD



ANTIFUNGAL SUSCEPTI BILITY TESTING
SENSITIVITY OF ANTIFUNGAL AGENTS





MASTER CHART

S.no	Name	Age	Sex	occupation	Address IP/OP no	Eye	Predisposing factors		KOH mount	Culture	sensitivity		
							Trauma	Others			Disk diffusion test	Agar dilution	Broth micro dilution
1.	Muniyan	64	M	Driver	4235/09	R			+	A.fumigatus	Ap, It,Vo	Ap,It	Ap,It
2.	Rangan	72	M	Merchant	4021/09	R			+	A.niger	It,Vo	Ap,It	Ap,It
3.	Kannappan	50	M	Pueon	4011/09	L	+		+	A.fumigatus	Ap,	Ap,It	Ap,It
4.	Chellammal	64	F	Vendor	4211/09	R							
5.	Kalimuthu	60	M	labour	4036/09	R	+		+	Penicillium	Ap, It,Vo	Ap,It	Ap,It
6.	Kandasamy	51	M	Typist	4020/09	R							
7.	Muniyandi	47	M	Watchman	4036/09	L		Post op	+	Fusarium	Ap, It,Vo	Ap,It	Ap,It
8.	Sankar	12	M	Driver	4321/09	L							
9.	Mohammedali	48	M	Mechanic	4017/09	R			+	A.niger	Ap, It,Vo	Ap,It	Ap,It
10.	Balasubramani	52	M	Security	4567/09	R			+	A.niger	Ap, It,Vo	Ap,It	Ap,It
11.	Chandrasekar	49	M	Fireman	4253/09	L							
12.	Vasanthi	42	F	Housewife	4364/09	L							
13.	Saravanan	18	M	Typist	4311/09	R							
14.	Subramani	54	M	Office boy	4125/09	L	+		+	A.fumigatus	Ap, It,Vo	Ap,It	Ap,It
15.	Marimuthu	50	M	Vendor	4138/09	L		Steroid	+	A.niger	It,Vo	Ap,It	Ap,It
16.	Jayamani	49	F	Farmer	4015/09	R			-	A.niger	Ap, It,Vo	Ap,It	Ap,It
17.	Kumar	42	M	Merchant	4112/09	L							
18.	Selvam	58	M	Grocer	4057/09	R			+	Fusarium spp	It,Vo	Ap,It	Ap,It
19.	Karuppiyah	60	M	Tea master	4023/09	R	+		+	Penicillium	Ap, It,Vo	Ap,It	Ap,It
20.	Sekar	38	M	Vendor	4006/09	L	+		+	Penicillium	It,Vo	It	Ap,It
21.	Anand	43	M	Painter	4036/09	L							
22.	Nalini	46	F	Labour	4176/09	R							
23.	Munusamy	33	M	Nursery	4001/09	R		Steroid	+	A.fumigatus	It,Vo	Ap,It	Ap,It
24.	Karpagam	41	F	Sweeper	4052/09	R		Native medicine	+	A.niger	Ap, It,Vo	Ap,It	Ap,It
25.	Sivakumar	56	M	Sports man	4113/09	R							

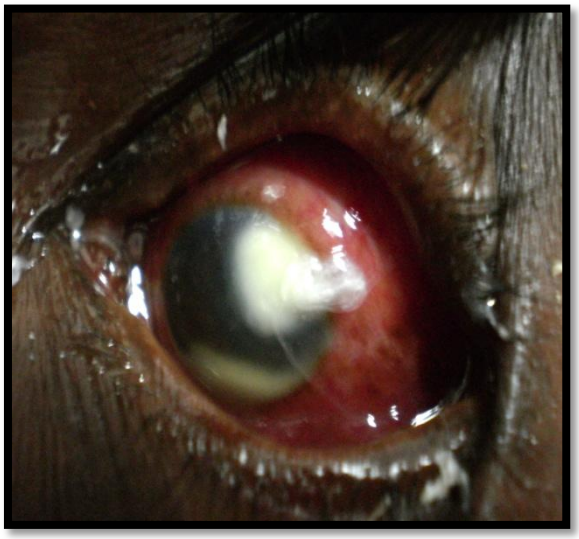
26.	Ayyappan	55	M	Milk man	4125/09	L		Leprosy	+	A.flavus	It,Vo	Vo	It
27.	Muniyandi	46	M	Broker	4154/09	R			+	A.niger	Ap, It,Vo	Ap,It	Ap,It
28.	Lakshmi	47	F	Housewife	4256/09	R			+	A.fumigatus	Ap,Vo	Ap,It	Ap,It
29.	Kandasamy	31	M	Electrician	4102/09	R			+	Fusarium	Ap, It,Vo	Ap,It	Ap,It
30.	Sengottayan	39	M	Waiter	4856/09	L			+	Penicillium	Ap, It,Vo	Ap,It	Ap,It
31.	Jagan	46	M	Cashier	4567/10	L							
32.	Sundaram	52	M	Laborer	4528/09	R							
33.	Kuppusamy	34	M	Lift operator	4532/09	R			+	A.niger	It,Vo	Ap,It	Ap,It
34.	Elumalai	31	M	Tailor	4235/09	L			+	Fusarium	Ap,	Ap,It	Ap,It
35.	Sadayappan	50	M	Baker	4453/09	L		Steroid	+	A.fumigatus	Ap,	Ap,It	
36.	Sarasammal	47	F	Nurse	4232/09	R			+	A.fumigatus	It,Vo		
37.	Navaneethan	42	M	Xerox shop	4435/09	L							
38.	Manikandan	37	M	Fisher man	4412/09	L			+	A.flavus	Ap, It,Vo	Ap,It	Ap,It
39.	Kanchana	62	F	Nursery	4475/09	R			+	Asp.niger	Ap, It,Vo	Ap,It	Ap,It
40.	Moorthy	66	M	Vendor	4321/09	L	+		+	Penicillium	Ap, It,Vo	Ap,It	Ap,It
41.	Karuppanan	38	M	Post man	4231/09	R	+		+	A.fumigatus	It,Vo	Ap,It	Ap,It
42.	Vetrivel	60	M	Bakery man	4356/09	R							
43.	Nancy	43	F	Shephard	4732/09	L			+	Penicillium	Ap,It,Vo	Ap,It	Ap,It
44.	Lakshmi	48	F	Cook	4766/09	L							
45.	Kesavan	42	M	Painter	4765/09	R	+		+	Candida	Ap,It,Vo,Fu		
46.	Elango	50	M	Courier man	4368/09	R							
47.	Anbarasu	24	M	Fisher man	4545/09	R			+				
48.	Anitha	57	F	Teacher	4234/09	R							
49.	Mariammal	60	F	Midwife	4631/09	L	+		+	Asp.niger	Ap, It,Vo	Ap,It	Ap,It
50.	Chidambaram	40	M	Labor	4539/09	R			+				
51.	Kalai	58	F	Office asst.	4586/09	R			+	Asp.niger	Ap, It,Vo	Ap,It	Ap,It
52.	Kanagavel	49	M	Hotel worker	4503/09	R							
53.	Ponnuraj	39	M	Fire man	4666/09	L	+		+	A.fumigatus	Ap,	Ap	Ap
54.	Dhinesh	16	M	Tasmac	4625/09	L			-	Curvularia	Ap, It,Vo	Ap,It	Ap,It
55.	Lalitha	51	F	Basket maker	4589/09	R			+	Penicillium	Ap, It,Vo	Ap,It	Ap,It
56.	Manickam	57	F	Flowerist	4725/09	R			+	Asp.niger	Ap, It,Vo	Ap,It	Ap,It
57.	Kanimozhi	46	F	Corp worker	4652/09	L							
58.	Manohar	67	M	Farmer	4622/09	L	+		+	Fusarium	Ap, It,Vo	Ap,It	Ap,It
59.	Chinnasamy	48	M	Cleaner	4276/09	R	+		+	A.flavus	Ap, It,Vo	Ap,It	Ap,It

60.	Kavitha	57	F	STD booth	4826/09	L							
61.	Nandhini	49	F	Cashier	4157/09	L							
62.	Radha	60	F	Nurse	4652/09	R			+	A.fumigatus	It,Vo	Ap,It	Ap,It
63.	Veerasamy	42	M	Conductor	4633/09	L	+		+	Penicillium	It,Vo	It	Ap,It
64.	Ponnuthai	52	F	astrologist	4587/09	R							
65.	Kaviyarasi	32	F	Dealer	4721/09	R							
66.	Murugan	50	M	Watch man	4764/09	L							
67.	Raja	21	M	Driver	4675/09	L							
68.	Rajeswari	54	F	Editor	4366/09	R			+	Penicillium	Ap, It,Vo	Ap,It	Ap,It
69.	Ramasamy	35	M	Gardener	4710/09	R	+		+	A.fumigatus	It,Vo		
70.	Latha	40	F	Typist	4725/09	R			+	A.fumigatus	Ap,It,Vo	Ap,It	Ap,It
71.	Ganeshan	49	M	Merchant	4756/09	R							
72.	Veerasamy	55	M	Lands man	4865/09	L		Post op	+	A.flavus	Ap, It,Vo	Ap,It	Ap,It
73.	Velmurugan	24	M	Office boy	4119/09	R							
74.	Palaniyappan	59	M	Hotel worker	4900/09	R	+		+	Acremonium	Ap,It,Vo	Ap,It	Ap,It
75.	Muniyandi	51	M	Mineworker	4888/09	R		Bells palsy	+	Penicillium	Ap, It,Vo	Ap,It	Ap,It
76.	Gayathri	54	F	Merchant	4853/09	L			+	Curvularia	Ap, It,Vo	Ap,It	Ap,It
77.	Geetha	44	F	Handloom	4656/09	L			+	Fusarium	Ap, It,Vo	Ap,It	Ap,It
78.	Karuppiyah	46	M	Carpenter	4178/09	R	+		+	A.fumigatus	Ap,	Ap,It	Ap,It
79.	Maheshwari	59	F	Cleaner	4528/09	R			+	A.flavus	Ap, It,Vo	Ap,It	Ap,It
80.	Murugan	23	M	Labour	4987/09	L			+	Candida	It,Vo,Fu		
81.	Chandran	56	M	Printer	4258/09	L							
82.	Gunavathy	39	F	Cook	4658/09	R	+		+	A.fumigatus	It,Vo	Ap,It	Ap,It
83.	Neelakandan	71	M	Tea master	4266/09	L							
84.	Veerasamy	55	M	Goldsmith	4895/09	L	+		+	A.fumigatus	Ap,	Ap,It	Ap,It
85.	Velmurugan	52	M	Driver	4674/09	R							
86.	Paneerselvam	69	M	Carpenter	4328/09	L			+	Curvularia	It,Vo	It	Ap,It
87.	Ganeshamurthy	54	M	Paper man	4936/09	R			+	A.flavus	It,Vo	It	It
88.	Govindhan	59	M	Bakery	4998/09	R							
89.	Sarasvathy	53	F	Housewife	4786/09	L	+		+	A.flavus	Ap,Vo	Ap,It	Ap,It
90.	Samiyappan	60	M	Cook	4528/09	L			+	A.fumigatus	Ap, It,Vo	Ap,It	Ap,It
91.	Pachaimuthu	57	M	School clerk	4625/09	R	+		+	Fusarium	It,Vo	It	Ap,It
92.	Sudalaimani	56	M	accountant	4913/09	R							
93.	Vetrivel	73	M	Police	4195/09	R							

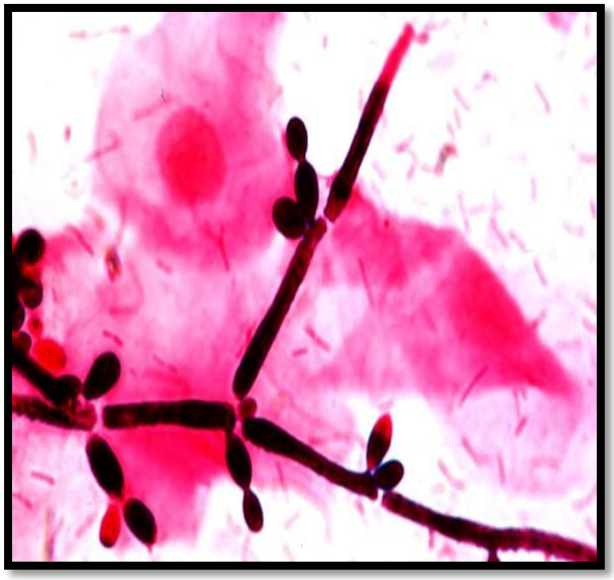
94.	Kannappan	51	M	Velding worker	4222/09	R	+		+	A.flavus	It,Vo	Ap,It	Ap,It
95.	Nachiyammal	54	F	Sweeper	4534/09	L	+		+	Penicillium	Ap, It,Vo	Ap,It	Ap,It
96.	Mariyappan	35	M	motorist	4982/09	R	+		+	A.flavus	Ap, It,Vo	Ap,It	Ap,It
97.	Poovannan	53	M	Politician	4625/09	R							
98.	Narayanan	60	M	Pawn broker	4677/09	R							
99.	Stella	26	F	Bed maker	4154/09	L			+	Penicillium	Ap, It,Vo	Ap,It	Ap,It
100.	Ponnurangan	46	M	Corp. worker	4751/09	L	+		+	Acremonium	Ap,It,Vo	Ap,It	Ap,It
101.	Selvaraj	37	M	Tailor	1651/10	R	+		+	A.fumigatus	It,Vo	Ap,It	Ap,It
102.	Padmavathy	43	F	Sweeper	1771/10	R	+		+	A.flavus	It,Vo	It	It
103.	Manohar	29	M	Conductor	1548/10	L							
104.	Sahul	44	M	Veldor	1265/10	L		Steroid	+	A.flavus	Ap, It,Vo	Ap,It	Ap,It
105.	Radhakrishnan	29	M	Dealer	1548/10	R							
106.	Karpagam	47	F	Labour	1586/10	L							
107.	Sathyamoorthy	50	M	Merchant	1254/10	L							
108.	Immanuvel	56	M	Draftsman	1586/10	R			+	A.fumigatus	It,Vo	Ap,It	Ap,It
109.	Nandhini	48	F	Vocalist	1365/10	L			+	A.fumigatus	Ap,	Ap,It	Ap,It
110.	Dhandayutham	34	M	Courier man	1254/10	R							
111.	Balan	54	M	Corp worker	1452/10	R			+	Penicillium	Ap, It,Vo	Ap,It	Ap,It
112.	Kavipriya	17	F	Flowerist	1652/10	L	+		+	Asp.niger	Ap, It,Vo	Ap,It	Ap,It
113.	Ranganathan	32	M	Sweeper	1466/10	L			+	A.flavus	Ap, It,Vo	Ap,It	Ap,It
114.	Karthikeyan	18	M	Porter	1563/10	R			+	Fusarium	Ap, It,Vo	Ap,It	Ap,It
115.	Kannammal	57	F	Servant maid	1774/10	R		Steroid	+	Penicillium	It,Vo	It	Ap,It
116.	Chinnasamy	36	M	clerk	1524/10	R							
117.	Venkatesan	24	M	Dhobi	1564/10	R	+		+	A.fumigatus	Ap,It	Ap,It	Ap,It
118.	Ponnambalam	33	M	carpenter	1666/10	L			+	Candida	Ap,It,Vo,Fu		
119.	Veena	50	F	Housewife	1447/10	R	+		+	A.flavus	Ap, It,Vo	Ap,It	Ap,It
120.	Somanathan	54	M	Handloom	1425/10	R	+		+	A.fumigatus	It,Vo	Ap,It	Ap,It
121.	Vetriselvi	59	F	Housewife	1352/10	R							
122.	Suganthi	49	F	Housewife	1425/10	L							
123.	Munusamy	56	M	sweeper	1653/10	L			+	Curvularia	Ap, It,Vo	Ap,It	Ap,It
124.	Tamaraiselvi	50	F	Tailor	1958/10	R	+		+	Fusarium	It,Vo	It	It
125.	Parameshwari	56	F	ventor	1547/10	R			+	Fusarium	It,Vo	It	It
126.	Eswaran	23	M	Auto driver	1654/10	L	+		+	A.flavus	Ap,It	Ap,It	Ap,It
127.	Rathna	58	F	Handloom	1456/10	L	+		+	A.fumigatus	Ap, It,Vo	Ap,It	Ap,It

128.	Sekar	28	M	Photograph	1478/10	R	+		+	Penicillium	It,Vo	It	Ap,It
129.	Gayathri	24	F	Painter	1547/10	L			+	A.flavus	Ap, It,Vo	Ap,It	Ap,It
130.	Baskaran	52	M	Barber	1598/10	L			+	A.fumigatus	It,Vo	It	It
131.	Jeevajothy	65	F	Servant maid	1574/10	R			+	Curvularia	Ap, It,Vo	Ap,It	Ap,It
132.	Kalaiselvi	51	F	Housewife	1532/10	L							
133.	Paneerselvam	35	M	Teacher	1685/10	R							
134.	Mayilvannan	80	M	Book seller	1654/10	R							
135.	Thanigaivel	68	M	Conductor	1687/10	L							
136.	Sumathy	14	F	Housewife	1685/10	L	+		+	A.flavus	Ap, It,Vo	Ap,It	Ap,It
137.	Diwakar	50	M	Builder	1362/10	R	+		+	A.fumigatus	It,Vo	Ap,It	Ap,It
138.	Ganeshamoorthy	38	M	Service man	1425/10	R							
139.	Kokila	27	F	Housewife	1754/10	R							
140.	Mohanraj	41	M	Vendor	1254/10	R	+		+	A.fumigatus	It,Vo	It	It
141.	Poongothai	61	F	Cook	1452/10	L			+	Penicillium	It,Vo	It	Ap,It
142.	Rathinam	66	F	Fisher man	1746/10	R		Steroid	+	A.flavus	Ap, It,Vo	Ap,It	Ap,It
143.	Ranjani	53	F	Dhobi	1953/10	R	+		+	A.fumigatus	Ap, It,Vo	It	It
144.	Rani	23	F	Housewife	1365/10	R							
145.	Anitha	72	F	AHN	1999/10	L							
146.	Leelavathy	55	F	Labour	2564/10	L		Native medicine	+	Acremonium	Ap,It,Vo	Ap,It	Ap,It
147.	Sasikala	37	F	Housewife	2451/10	R							
148.	Lakshmi	68	F	Milk man	2315/10	R							
149.	Selvi	25	F	Housewife	2147/10	L	+		+	A.flavus	Ap, It,Vo	Ap,It	Ap,It
150.	Christophy	31	F	Housewife	2854/10	L							
151.	Sripriya	51	F	Hotel worker	2654/10	R							
152.	Vani	35	F	Vegetable shop	2856/10	L		Post op	+	Curvularia	It,Vo	Ap,It	Ap,It
153.	Suseela	52	F	Labour	2754/10	L	+		+	A.fumigatus	Ap, It,Vo	It	It
154.	Nandhini	27	F	Tailor	2311/10	R							
155.	Kousalya	21	F	Teacher	2455/10	L							
156.	Poovarasi	48	F	Worker	2862/10	R							
157.	Ponnathal	42	F	Labour	2645/10	R							
158.	Mariyathal	66	F	Cleaner	2763/10	L			-	Candida	Ap,It,Vo,Fu		
159.	Mahalakshmi	24	F	Employee	2648/10	L							
160.	Poovatal	33	F	Merchant	2687/10	R							

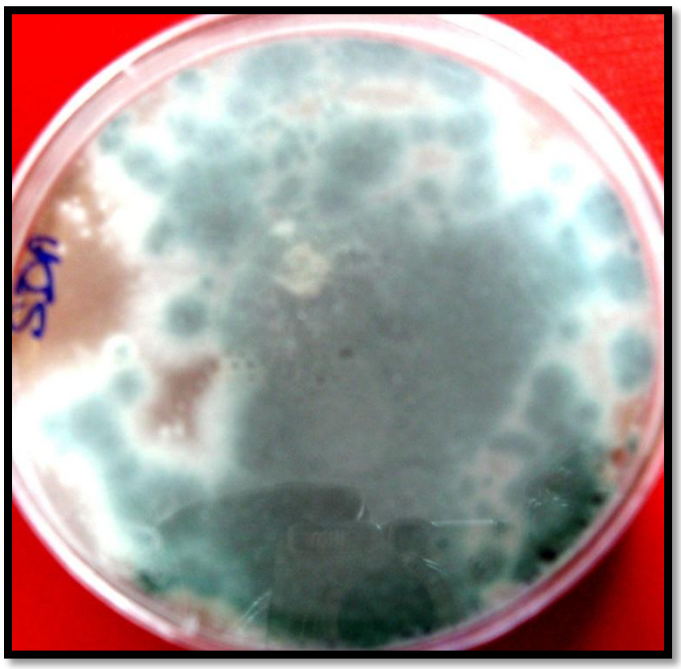
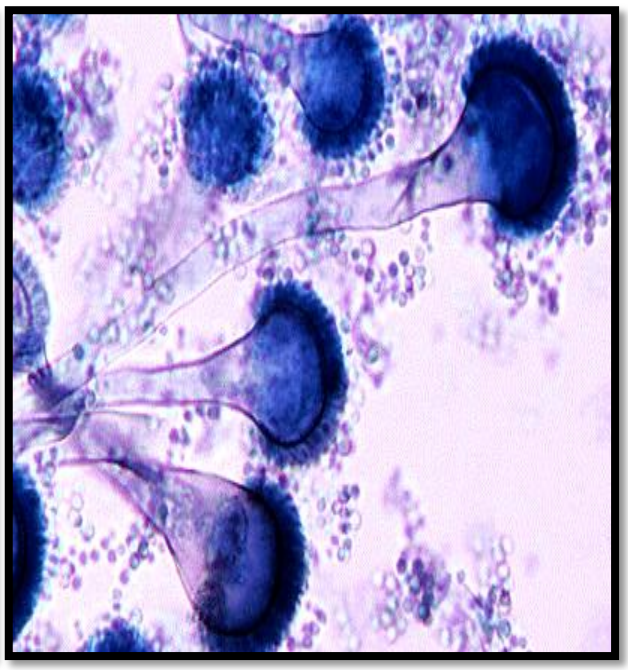
A CASE OF CORNEAL ULCER AND SPECIMEN TAKEN BY SCRAPING



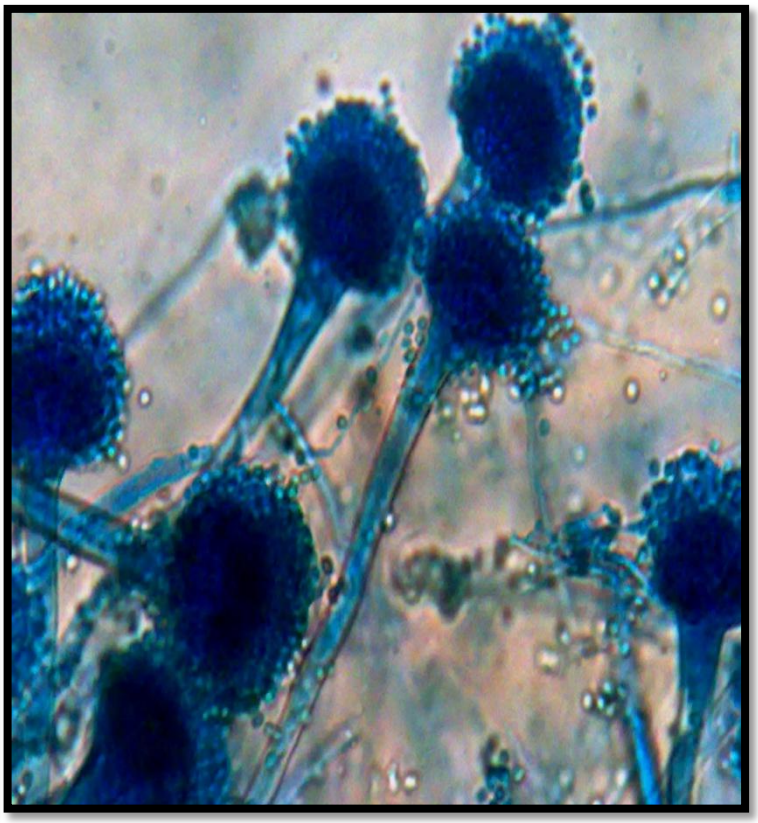
10% KOH MOUNT (FUNGAL ELEMENT) AND GRAM STAIN (YEAST CELLS)



ASPERGILLUS FUMIGATUS ON SDA – LPCB MOUNT

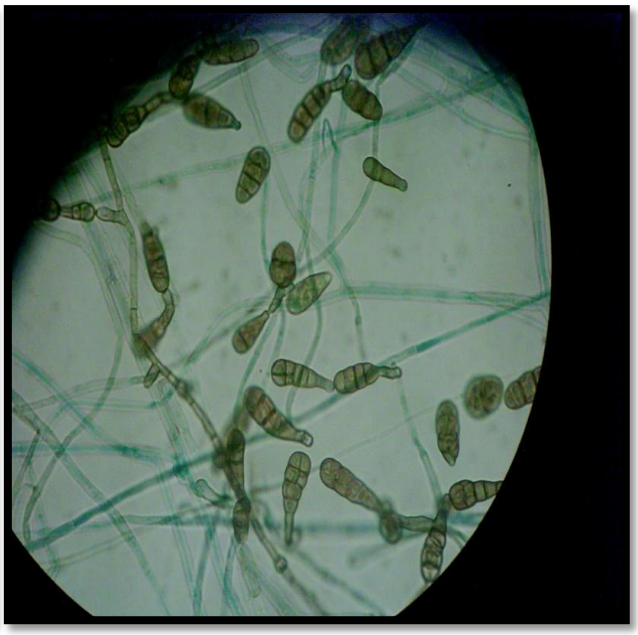



ASPERGILLUS NIGER ON SDA – LPCB MOUNT

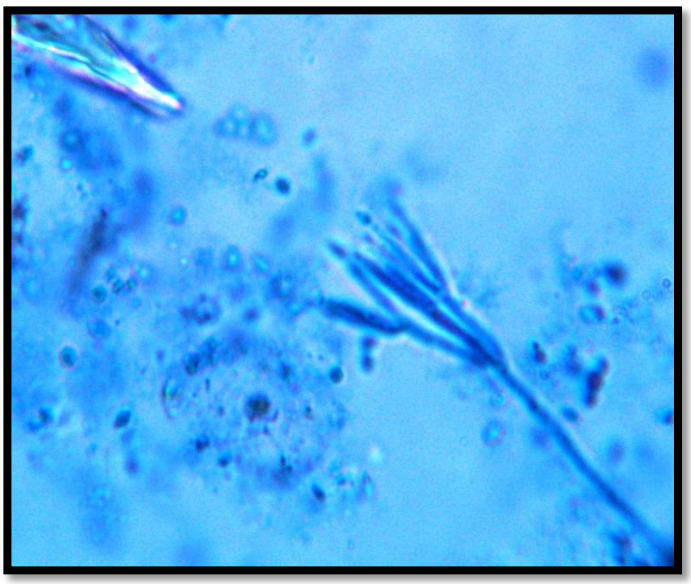



FUSARIUM ON SDA – LPCB MOUNT

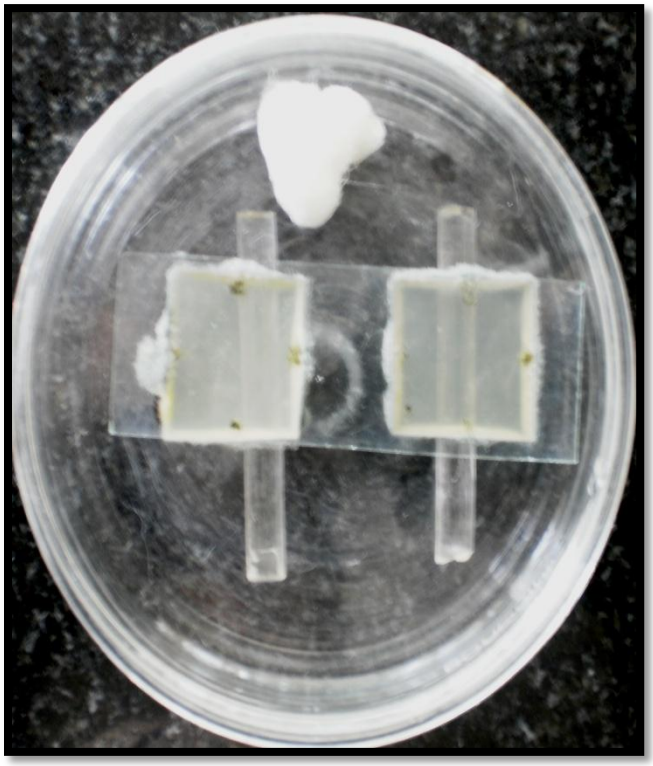
CURVULARIA ON SDA – LPCB MOUNT



PENICILLIUM ON SDA – LPCB MOUNT



SLIDE CULTURE



ANTIFUNGAL SUSCEPTIBILITY TESTING

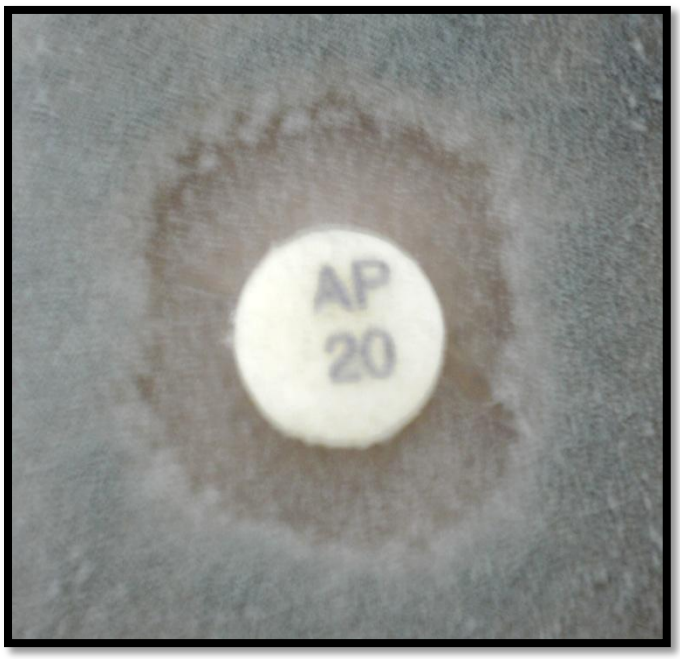
DISK DIFFUSION TEST

Ap : Amphotericin B It : Itraconazole Fu : Fluconazole

DISK DIFFUSION TEST

AMPHOTERICIN RESISTANCE BY PENICILLIUM

(ZONE SIZE <15MM)

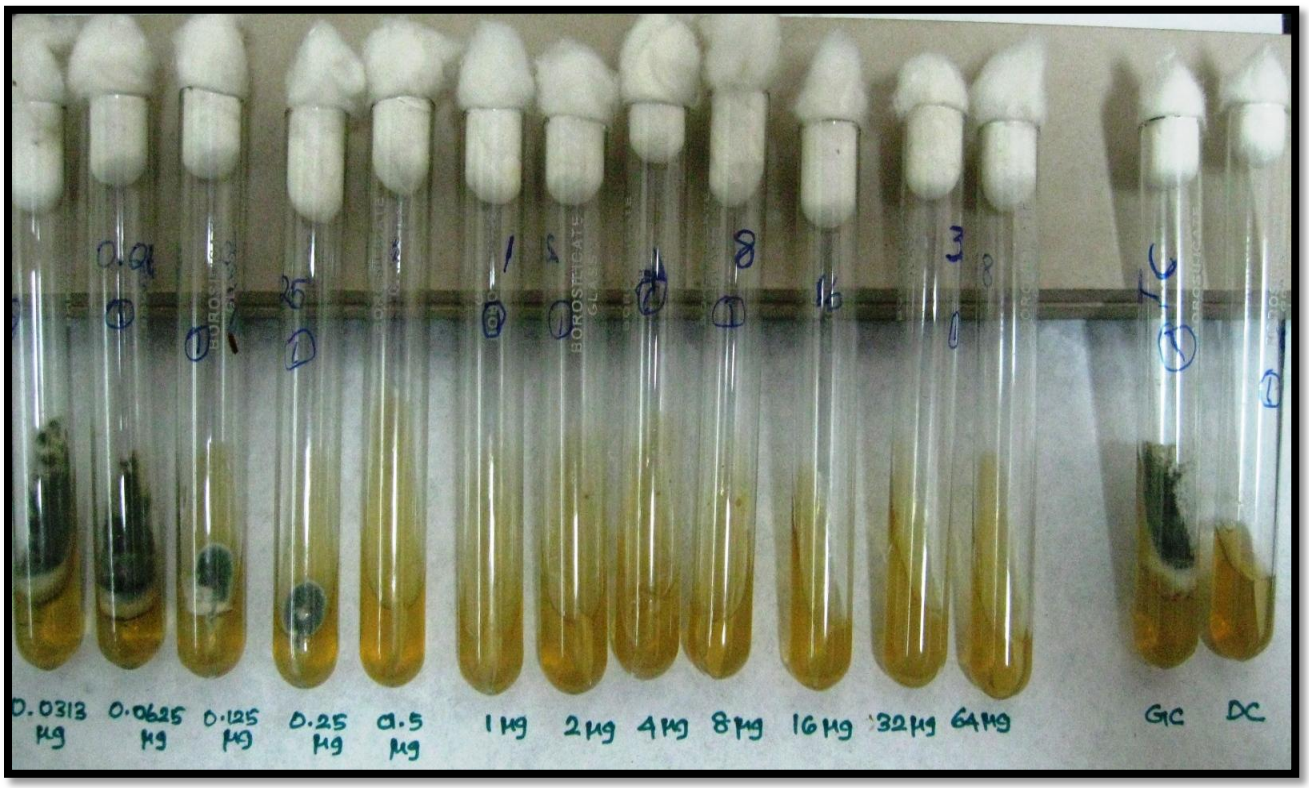


DISK DIFFUSION TEST

FLUCONAZOLE RESISTANCE

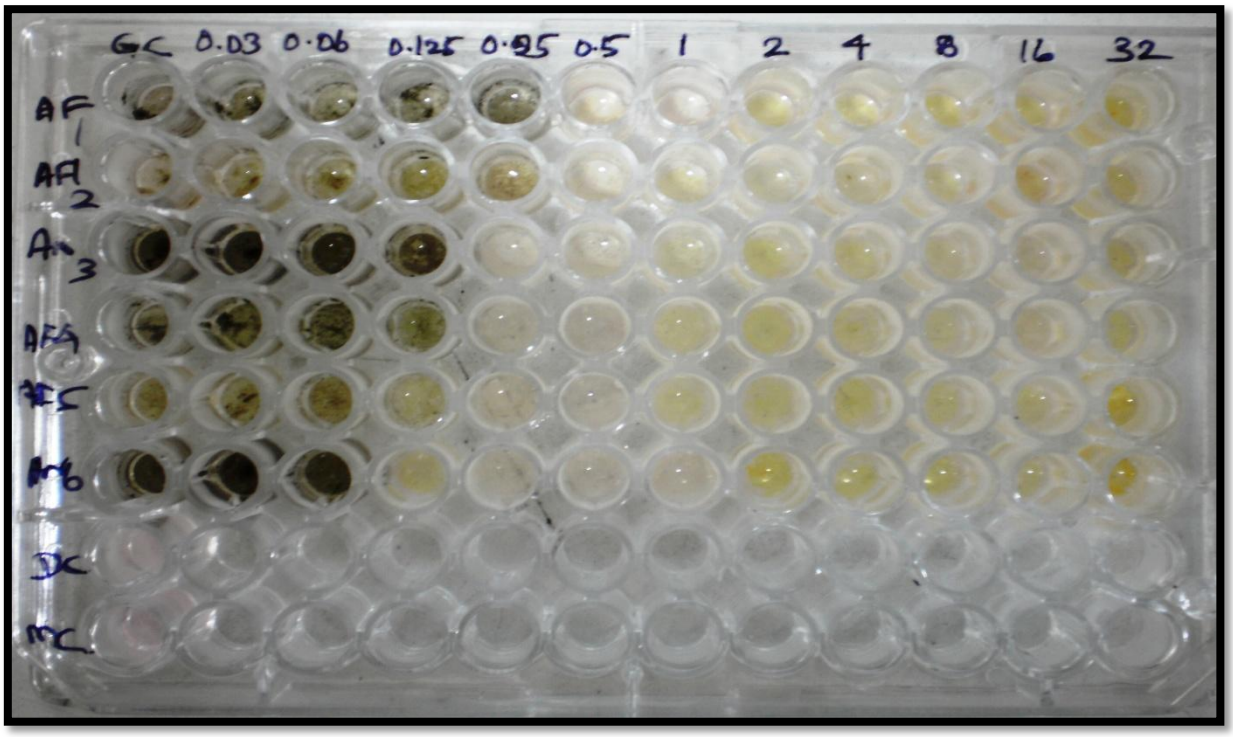
ASPERGILLUS FUMIGATUS

AGAR DILUTION METHOD (MIC) FOR PENICILLIUM SPECIES



AGAR DILUTION METHOD (MIC) FOR FUSARIUM SPECIES

BROTH MICRODILUTION METHOD (MIC)



MIC determination of Aspergillus species